The
Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. 6 *November 26, 1909* No. 5

THE FECAL BACTERIA OF HEALTHY MEN.*

PART II. QUANTITATIVE CULTURE EXPERIMENTS.

WARD J. MACNEAL, LENORE L. LATZER, AND JOSEPHINE E. KERR.

(From the Laboratory of Physiological Chemistry, Department of Animal Husbandry, University of Illinois, Urbana, Ill.)

CONTENTS.

INOCULATION MATERIAL.—Quantitative suspension of the mixt fecal flora. Suspension of bacterial spores. The value of quantitative culture methods.

BACTERIA GROWN ON PLATE CULTURES.—Aerobic and anaerobic agar. Aerobic gelatin. Species present. Number of cultivable facultative anaerobes. Variations. Aerobic spores. Anaerobic spores. Species. Variations in number.

RELATION OF CULTIVABLE BACTERIA TO TOTAL FECAL BACTERIA.—Dead bacterial cells. Species cultivable by special methods. Obligate parasites.

FERMENTATION TUBES.—Gas production in sugar broth. Variations. Bacteria in the sediments. Limitation of gas production. Milk fermentation tubes inoculated with mixt flora; with spore material. Blood broth inoculated with spore material.

CONCLUSIONS.

REFERENCES.

INOCULATION MATERIAL.

THE homogeneous suspension of the mixt feces, containing one part of feces in one hundred parts of suspension, was employed as the source of material † for all the culture experiments. This original

* Received for publication August 8, 1909.

† See Part I, this *Journal*, 1909, 6, p. 126.

suspension was called No. 1. One c.c. of it contained the bacteria of 10 mg. of the fresh feces. From it a series of dilutions was prepared and numbered as follows:

Suspension No. 2, 1 to 1,000, 1 c.c. represents 1 mg. feces.
Suspension No. 3, 1 to 10,000, 1 c.c. represents 0.1 mg. feces.
Suspension No. 4, 1 to 100,000, 1 c.c. represents 0.01 mg. feces.
Suspension No. 5, 1 to 1,000,000, 1 c.c. represents 0.001 mg. feces.

Each suspension was thoroughly mixt as prepared, and again each time before measuring out portions from it. A portion of Suspension No. 2 was transferred to a test tube and heated in a water bath at 80° C. for 15 minutes and then cooled. By this means the vegetative bacteria were killed and the resulting inoculation material was designated as Suspension No. 2, Spores. This procedure of quantitative dilution of the feces we consider to be of special value in making cultures, as it is then possible to estimate the number of cultivable bacteria demonstrated by any particular culture method upon a definite quantity of the original feces. The results of cultures thus become quantitatively comparable one with another and also with the direct microscopic examinations. It seems to us very desirable that quantitative methods of this kind should be generally adopted and employed in estimating the importance of any species in the fecal flora.

The variety of bacteria which may occur in the human feces is almost endless, but the species of real significance are limited in number. The accidental, unimportant bacterial species are represented by relatively few individuals, but may assume a very prominent place in cultures made without regard to quantitative methods. Even among the really significant members of the fecal flora, quantitative culture methods will be necessary to determine the real importance of the various species. In the classical work upon fecal bacteria it has generally been assumed that the relative importance of the different species in artificial cultures is a direct indication of their relative importance in the feces. The fallacy of this assumption is illustrated by the work of Escherich[5] and of Tissier[17] on the fecal flora of the breast fed infant. Thus Escherich, studying the fecal flora of the nursling by the gelatin plate method, concluded that *B. lactis aerogenes* and *B. coli* make up practically the entire bacte-

rial content of the breast fed infant's stool. Tissier, employing a different culture method, deep tubes of glucose agar, concluded that an entirely different microorganism, *B. bifidus communis*, makes up practically the whole flora of the breast fed infant's stool. Each of these observers carried out most careful direct microscopic examinations of the feces in conjunction with his culture experiments and found the bacteria cultivated to be morphologically identical with those in the original material. Tissier's work following that of Escherich clearly disproves Escherich's conclusion, but it is not impossible that some new culture method may bring to development a new species of bacteria occurring in still greater quantity in the nursling's stool than even *B. bifidus*. Tissier's conclusion that the species giving rise to the greatest number of colonies in deep glucose agar tubes is identical with the dominant microorganism seen microscopically in the nursling's feces, even tho rendered probable by the similarity of the two in morphology and staining properties, will be made certain only when it is shown that the number of colonies of this bacillus, developed from a definite quantity of feces, bears some approximate relation to the number of bacilli of this type counted microscopically in the same quantity of the feces. Escherich clearly recognized the source of error in interpreting the results of culture experiments and the discrepancy between them and the results of direct microscopic examination. Thus he* says: "Dabei stellten sich alsbald wesentliche Unterschiede in dem Ergebniss beider Untersuchungsmethoden heraus in der Art, dass die Zahl und Mannigfaltigkeit der im microscopischen Bilde vorhandenen Bakterien eine erheblich grössere war als diejenige der in der Cultur erhaltenen. Trotz aller Variationen des Nährbodens blieb das Resultat das gleiche und ich musste mir sagen, dass wenigstens mit den von mir ausschliesslich angewandten Methoden des festen Nährbodens das Ergebniss der Cultur nur im *positiven, niemals im negativen* Sinne entscheidend sein könne. Das microscopische Bild wird uns den festen, objectiven Rahmen liefern müssen, in welchen wir die zunächst noch unvollständigen Ergebnisse der Culturmethoden einzureihen haben." Tissier also observed bacterial forms in his microscopic preparations of feces which he was unable to bring to development

* *Darmbakterien des Säuglings*, p. 12.

in culture, altho in some places he speaks of the deep glucose agar tube as a universal culture method.*

The postulate so clearly enunciated by Escherich is certainly the safe rule. It does not seem probable that any single culture method will ever be devised which will bring to development all the living microorganisms present in the human feces, or even be equally favorable to the several different species which may grow upon it. More definite and certain progress will be made by culture methods which are frankly recognized as bringing to development certain definite species or groups of species to the exclusion of others, applied in a quantitative manner, the results of which may be directly compared with each other and with the results of direct microscopic examination of the original material. The results of our culture experiments are essentially incomplete and having been carried out quantitatively this incompleteness is clearly evident. We consider them therefore of peculiar value in connection with the direct quantitative observations recorded in Part I, with which they may be quantitatively compared.

PLATE CULTURES.

Three sets of plate cultures were made from the unheated material: (1) upon litmus lactose agar at 37° C. in the presence of air, (2) upon litmus glucose agar at 37° C. in an atmosphere of hydrogen, and (3) upon litmus lactose gelatin at 18° C. in the air. In preparing these plates, portions of 0.25 c.c. of Suspension 3, and 0.50 c.c. of Suspensions 4 and 5 were measured out into sterile Petri dishes. The agar was melted and cooled to 45° C. in a constant temperature water bath. Five drops of a sterile strong solution of purified litmus† were added to each tube of medium, the contents of the tube then added to the bacterial suspension in the Petri dish, covered, and thoroughly mixt, and then allowed to solidify in a level place. The gelatin was also colored with the litmus after it was melted ready to pour into the Petri dishes. When solidified, the lactose agar plates were transferred to the incubator and allowed to develop for 24 hours. The colonies on each plate were then counted and the number of bacteria

* "Elle est en effet absolument générale," Thèse, p. 41; and "Si nous avons insisté sur cette méthode, d'isolement c'est qu'elle nous parait absolument générale," Thèse, p. 43.

† We have found Merck's Highest Purity Litmus to be satisfactory.

per milligram feces represented by them estimated. The glucose agar plates, after solidification, were placed in a Novy anaerobe jar, stacked upon a low glass tripod (see Fig. 1). Upon the bottom of the jar, underneath the plates, a small amount (about 2 gm.) of pyrogallic acid was placed, and on top of the stack of plates, a small (50 c.c.) flask filled with strong sodium hydroxide solution. The flask (see Fig. 2) was provided with a syphon spout to the end of which a rubber tube was attached, leading down at the side of the stack of Petri dishes to the bottom of the jar. A small tube containing glucose gelatin, colored with a few drops of methylene blue and then steamed for a short time, was also placed in the jar to serve as an indicator control of the anaerobiosis, the leuko-methylene blue

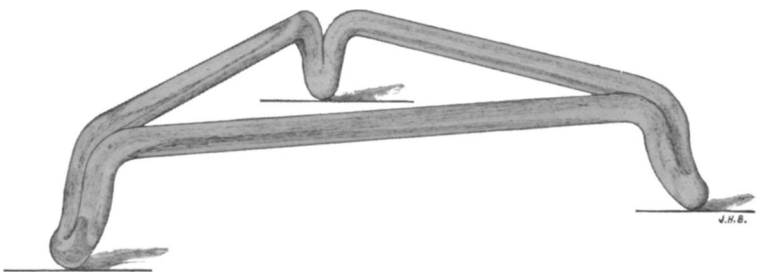

FIG. 1

remaining reduced and colorless in the absence of oxygen. The cover of the jar was then clamped on, and hydrogen passed through it for several hours. The stop-cock was then closed, the jar disconnected from the hydrogen generator, and inclined slightly to start the flow of alkali through the siphon spout from the flask down onto the pyrogallic acid in the bottom of the jar. In this way the pyrogallic acid became available for absorption of the last traces of oxygen. The jar was then placed in the incubator at 37° C. for 48 hours, after which the colonies were counted and the bacteria per milligram feces calculated. The gelatin plates were allowed to develop for four days at 18°–20° C., after which the colonies were counted and the bacteria per milligram feces calculated. The reaction of the colonies on the litmus medium was noted in each case. On the lactose (aerobic) agar, alkaline colonies were frequently observed, generally corresponding in number to the alkaline colonies develop-

ing on the gelatin plates from the same stool. All the colonies developing on the anaerobic glucose agar plates were invariably acid in reaction, but from time to time colonies producing an unusually intense red color were noted. The gelatin plates permitted an immediate separation of the colonies into four kinds, according to reaction and liquefaction, as well as producing more definitely recognizable types of colonies. The relative numbers of different colonies were noted here in addition to the total colonies.

Fig. 2

Table 8 shows the number of colonies developed on the aerobic agar and anaerobic agar plates from 202 different stools, and the number of colonies developed on the gelatin plates from 144 stools, and in these latter the relative numbers of liquefying and non-liquefying, acid and alkaline colonies. These results present nothing new in themselves, being essentially the same as obtained by a large number of investigators. They are, however, of particular importance in connection with the other examinations made upon the same stools, especially the direct quantitative examinations reported in Part I.

The number of colonies on these three sets of plate cultures, aerobic litmus lactose agar, anaerobic litmus glucose agar, and aerobic litmus lactose gelatin, generally in close agreement, represents approximately the quantity of cultivable *B. coli* in the feces. Other bacteria occurred in small numbers and not constantly. The smallest number of colonies per milligram feces was observed in Subject G, Dec. 14, 1,360 on the aerobic agar, but 6,440 on the anaerobic agar. Subject D, April 27, shows 2,420 on the aerobic agar, 2,900 on the anaerobic agar, and 3,140 on the gelatin plates. The total number of bacteria by direct microscopic counts upon these stools was 179,000,000 and 252,000,000 bacteria per milligram respectively. The highest colony count occurs in Subject F, June 8, 1,500,000* per milligram on the aerobic agar plates. The anaerobic agar plates show 1,100,000, but the gelatin plates only 380,000 per milligram. The direct count on this sample of feces was 477,000,000 bacteria per milligram. Subject E, Mar. 2, shows 1,200,000 colonies per milligram on all three sets of plates. The direct count in this case was 480,000,000 bacteria per milligram. Of the 12 individuals, Subject E shows the highest average value, 280,000 colonies per milligram feces, Subject J, the lowest, 33,000. The average of the direct counts for these subjects were 424,000,000 and 328,000,000 bacteria per milligram feces. There is moreover a remarkable variation in the number of cultivable *B. coli* in different stools of the same individual, and this variation seems to bear no constant relation to the total number of bacteria present, nor to the dryness of the stool, altho these factors appear to have an influence in some cases. In Table 9, the maximum, mean, and minimum number of colonies developed on the plates, and of bacteria counted directly, are given, summarized from Tables 8 and 2. The extreme individual variations are here clearly shown.

Sucksdorff,[15] who first noted this remarkable variation in the number of cultivable bacteria in the human feces, ascribed it to the variation in the number of bacteria swallowed with the food. By taking only sterilized food he reduced very considerably the number of colonies developed from the feces. Very serious errors in Sucksdorff's

* Attention should be called to the fact that inasmuch as the same dilutions were employed in making the plates from each stool, these high counts are really too low, because of inhibition of development by crowding on the plates.

TABLE 8.

Bacteria per Milligram Feces, Developed into Colonies on Plate Cultures.

SUBJECT A

Laboratory No.	Date	Unheated Suspension								Spore Material (Heated at 80° C. 15 Minutes)			Microscopic Count
				Aerobic Gelatin							Anaerobic Blood Agar		
		Aerobic Agar	Anaerobic Agar	Total Colonies	Bacteria				Molds	Aerobic Agar	Colonies	Types	
					Non-liq.		Liquefying						
					Acid P. ct.	Alk. P. ct.	Acid. P. ct.	Alk. P. ct.	P. ct.				
B 37	Nov. 12	17,400	19,000	.....	....	...	...	...	...	..	....		336,000,000
B 60	Nov. 26	7,100	10,000	.....	....	...	...	...	...	..	....		306,000,000
B 83	Dec. 10	20,200	21,400	.....	....	...	...	...	...	..	....		358,000,000
B 110	Jan. 6	21,200	27,600	6,400	97.0	3.0	...	...	...	..	....		244,000,000
B 122	Jan. 22	15,600	16,200	16,740	100.0	0.0	0.0	0.0	0.0	..	....		339,000,000
B 134	Feb. 6	52,680	51,322	58,200	98.0	2.0	0.0	0.0	0.0	..	....		348,000,000
B 146	Feb. 24	11,100	16,780	10,800	99.0	1.0	0.0	0.0	0.0	..	4,497	I	348,000,000
B 158	Mar. 11	50,690	31,766	27,182	100.0	0.0	0.0	0.0	0.0	..	915	I	447,000,000
B 170	Mar. 27	19,720	18,020	19,300	98.0	2.0	0.0	0.0	0.0	12	632	I–II	472,000,000
B 182	April 10	14,620	14,380	13,340	100.0	0.0	0.0	0.0	0.0	23	not counted	II–III	407,000,000
B 194	April 27	38,674	30,500	22,760	99.0	1.0	0.0	0.0	0.0	7	97	I–II	478,000,000
B 206	May 13	64,628	37,771	60,000	100.0	0.0	0.0	0.0	0.0	4	367	I–IV	359,000,000
B 218	May 28	20,000	19,844	23,970	100.0	0.0	0.0	0.0	0.0	17	8,467	I–III–VIII	460,000,000
B 230	June 15	52,445	57,528	50,497	100.0	0.0	0.0	0.0	0.0	6	345	I–II–III	539,000,000
B 242	July 1	47,801	51,399	60,420	100.0	0.0	0.0	0.0	0.0	24	309	III–V–VIII	472,000,000
B 254	July 17	94,691	57,642	90,860	100.0	0.0	0.0	0.0	0.0	46	13,698	I–III	347,000,000
B 266	July 31	57,200	55,763	.....	Plates not counted				...	3,556	6,625	I–VI	441,000,000
Average		35,632	31,583	35,421	99.30	0.70	0.00	0.00	0.00	411	3,595		394,000,000
Minimum		7,100	10,000	6,400	97.00	0.00	0.00	0.00	0.00	4	97		244,000,000
Maximum		94,691	57,642	90,860	100.0	3.00	0.00	0.00	0.00	3,556	13,698		539,000,000

SUBJECT B.

B 33	Nov. 10	39,000	30,000	.....	....	...	...	...	...	..	..		215,000,000
B 56	Nov. 22	229,000	38,000	.....	....	...	...	...	...	..	..		309,000,000
B 79	Dec. 9	324,000	309,000	.....	....	...	...	...	...	..	..		223,000,000
B 102	Dec. 26	33,557	40,300	.....	....	...	...	...	...	..	..		151,000,000
B 114	Jan. 12	109,750	108,650	98,060	99.0	1.0	0.0	0.0	0.0	..	..		248,000,000
B 126	Jan. 27	65,320	57,200	72,200	92.0	0.0	0.0	8.0	0.0	..	42	Not determined	325,000,000
B 138	Feb. 13	119,940	104,900	97,820	100.0	0.0	0.0	0.0	0.0	..	88	I	239,000,000
B 150	Mar. 2	747,000	658,160	736,440	100.0	0.0	0.0	0.0	0.0	..	385	I	284,000,000
B 162	Mar. 16	29,800	19,980	41,840	99.8	0.1	0.1	0.0	0.0	..	75	I	296,000,000
B 174	April 1	64,760	47,760	39,360	98.0	2.0	0.0	0.0	0.0	22	54	I–II	366,000,000
B 186	April 16	82,080	43,340	103,180	99.9	0.1	0.0	0.0	0.0	51	75	I–II	416,000,000
B 198	May 1	17,400	13,960	16,540	99.0	1.0	0.0	0.0	0.0	10	55	I–II	379,000,000
B 210	May 18	162,100	158,560	180,840	100.0	0.0	0.0	0.0	0.0	8	6	VII	296,000,000
B 222	June 3	55,100	47,840	64,100	100.0	0.0	0.0	0.0	0.0	21	36	I–III	298,000,000
B 234	June 19	78,500	69,580	88,100	100.0	0.0	0.0	0.0	0.0	29	47	I–VIII	380,000,000
B 246	July 6	14,960	9,840	9,160	100.0	0.0	0.0	0.0	0.0	9	964	I–IX	425,000,000
B 258	July 22	103,280	86,460	108,760	99.8	0.0	0.2	0.0	0.0	7	28	I–IX	269,000,000
Average		133,856	108,443	127,415	99.04	0.32	0.02	0.62	0.00	20	155		301,000,000
Minimum		14,960	9,840	9,160	92.00	0.00	0.00	0.00	0.00	7	6		151,000,000
Maximum		747,000	658,160	736,440	100.00	2.00	0.20	8.00	0.00	51	964		425,000,000

SUBJECT C.

B 39	Nov. 13	1,787	10,800	.....	....	...	...	...	...	..	..		341,000,000
B 62	Nov. 26	4,780	13,946	.....	....	...	...	...	...	..	..		673,000,000
B 85	Dec. 12	27,320	326,000	.....	....	...	...	...	...	..	..		438,000,000
B 104	Dec. 28	15,106	11,380	.....	....	...	...	...	...	..	..		359,000,000
B 116	Jan. 14	45,413	42,142	32,060	100.0	0.0	0.0	0.0	0.0	..	..		405,000,000
B 128	Jan. 29	54,533	37,708	51,652	99.8	0.1	0.1	0.0	0.0	..	64	I–X	402,000,000
B 140	Feb. 20	359,924	310,161	272,479	100.0	0.0	0.0	0.0	0.0	..	449	I	457,000,000
B 154	Mar. 6	7,400	8,893	7,780	83.0	0.0	0.0	0.0	17.0	..	10	I–XI	463,000,000
B 166	Mar. 23	3,160	4,160	.....	Plates lost		by melting		...	..	8	I	436,000,000
B 178	April 6	22,760	15,360	24,980	100.0	0.0	0.0	0.0	0.0	17	Plates not counted	I	375,000,000
B 190	April 22	9,100	28,900	8,900	97.0	0.0	0.0	0.0	3.0	6	124	II–XII	461,000,000
B 202	May 7	49,635	45,161	63,600	100.0	0.0	0.0	0.0	0.0	16	97	I–II–XIII	561,000,000
B 214	May 23	46,058	29,760	45,705	99.0	0.0	0.0	0.0	1.0	12	44	I	457,000,000
B 226	June 8	57,007	44,227	61,494	99.8	0.2	0.0	0.0	0.0	Plates not counted	19	I	439,000,000
B 238	June 25	681,728	680,737	760,604	100.0	0.0	0.0	0.0	0.0	538	56	VIII	678,000000
B 250	July 13	152,408	157,634	432,907	100.0	0.0	0.0	0.0	0.0	Plates not counted	41	I–VIII	499,000,000
B 262	July 27	223,840	153,130	228,960	100.0	0.0	0.0	0.0	0.0	13	4	I	388,000,000
Average		103,645	112,947	165,927	98.21	0.03	0.01	0.00	1.75	100	83		461,000,000
Minimum		1,787	4,160	7,780	83.00	0.00	0.00	0.00	0.00	6	4		341,000,000
Maximum		681,728	680,737	760,604	100.00	0.20	0.10	0.00	17.00	538	449		678,000,000

TABLE 8—*Continued.*

SUBJECT D.

Laboratory No.	Date	Unheated Suspension								Spore Material (Heated at 80° C. 15 Minutes)			Microscopic Count
		Aerobic Agar	Anaerobic Agar	Aerobic Gelatin						Aerobic Agar	Anaerobic Blood Agar		
				Total Colonies	Bacteria				Molds		Colonies	Types	
					Non-liq.		Liquefying						
					Acid P. ct.	Alk. P. ct.	Acid P. ct.	Alk. P. ct	P. ct.				
B 34	Nov. 11	15,000	20,000	.....	....	...	...	...	...	..	..		348,000,000
B 57	Nov. 23	70,000	58,000	.....	....	...	...	...	...	..	..		448,000,000
B 80	Dec. 10	14,000	15,000	.....	....	...	...	...	...	..	..		289,000,000
B 99	Dec. 23	8,907	10,200	.....	....	...	...	...	...	..	..		387,000,000
B 123	Jan. 25	23,200	20,520	27,980	100.0	0.0	0.0	0.0	0.0	..	1,991	I	262,000,000
B 135	Feb. 10	7,620	8,000	7,720	100.0	0.0	0.0	0.0	0.0	..	0		288,000,000
B 147	Feb. 27	38,740	42,010	38,160	99.9	0.1	0.0	0.0	0.0	..	2	I	270,000 000
B 157	Mar. 11	6,080	6,500	3,960	98.0	2.0	0.0	0.0	0.0	..	2	Facultative	352,000,000
B 169	Mar. 27	13,560	12,920	13,920	100.0	0.0	0.0	0.0	0.0	10	7	Facultative	322,000,000
B 181	April 10	37,440	38,780	40,660	99.9	0.0	0.1	0.0	0.0	23	0		308,000,000
B 193	April 27	2,420	2,900	3,140	99.0	1.0	0.0	0.0	0.0	14	54	XIV	252,000,000
B 205	May 13	15,800	10.460	15,480	100.0	0.0	0.0	0.0	0.0	3	3	Facultative	328,000,000
B 217	May 28	32,080	34,000	30,260	100.0	0.0	0.0	0.0	0.0	9	3	XV	330,000,000
B 229	June 15	15,840	14,540	17.360	100.0	0.0	0.0	0.0	0.0	12	7	XV	265,000,000
B 241	July 1	74,000	61,920	87,135	100.0	0.0	0.0	0.0	0.0	9	0		193,000,000
B 253	July 17	41,740	35,340	41,200	100.0	0.0	0.0	0.0	0.0	7	3,538	I–VIII	307,000,000
B 265	July 31	36,400	28,800	36,378	99.0	0.0	0.0	0.0	1.0	121	695	I	304,000,000
Average		26,637	24,699	27,950	99.67	0.24	0.01	0.00	0.08	23	485		305,000,000
Minimum		2,420	2,900	3.140	98.00	0.00	0.00	0.00	0.00	3	0		193,000,000
Maximum		74,000	61,920	87,135	100.00	2.00	0.10	0.00	1.00	121	3,538		448,000,000

SUBJECT E.

B 41	Nov. 14	113,950	102,000	.....	....	...	...	...	...	..	..		254,000,000
B 64	Nov. 27	139,000	147,000	.....	....	...	...	...	...	..	..		279,000,000
B 87	Dec. 13	170,000	136,000	.....	....	...	...	...	...	..	..		365,000,000
B 106	Dec. 30	153,000	171,000	.....	....	...	...	...	...	..	..		431,000,000
B 118	Jan. 16	151,000	231,000	.....	....	...	...	...	...	..	..		454,000,000
B 130	Jan. 31	30,600	36,000	41,500	100.0	0.0	0.0	0.0	0.0	..	292	I	494,000,000
B 142	Feb. 19	221 833	165,236	171,600	100.0	0.0	0.0	0.0	0.0	..	57	I	308,000,000
B 149	Mar. 2	1,200,213	1,162,273	1,232,677	100.0	0.0	0.0	0.0	0.0	..	33	I–XVI	480,000,000
B 161	Mar. 16	449,231	362,066	377,651	100.0	0.0	0.0	0.0	0.0	..	303	I	299,000,000
B 173	April 1	123,431	117,007	135,337	100.0	0.0	0.0	0.0	0.0	20	118	I	535,000,000
B 185	April 16	290,920	224,423	229,058	100.0	0.0	0.0	0.0	0.0	4	188	I–II–III–XVII	441,000,000
B 197	May 1	142,312	133,176	189,848	100.0	0.0	0.0	0.0	0.0	17	798	I–III–XVIII	514,000,000
B 209	May 18	99,310	92,144	94,714	100.0	0.0	0.0	0.0	0.0	7	17	I–XIX	513,000,000
B 221	June 3	833,561	846,115	107,271	100.0	0.0	0.0	0.0	0.0	35	13	I–III–VIII	343,000,000
B 233	June 19	232,271	224,742	269,281	100.0	0.0	0.0	0.0	0.0	21	80	I–III	468,000,000
B 245	July 6	96,864	100,080	130,320	100.0	0.0	0.0	0.0	0.0	3	3,152	I–VIII	373,000,000
B 257	July 22	299,074	322,108	391,750	99.0	0.0	0.0	0.0	1.0	34	18	I–IX–XX	569,000,000
Average		279,210	268,963	280,017	99.92	0.00	0.00	0.00	0.08	18	422		424,000,000
Minimum		30,600	36,000	41,500	99.00	0.00	0.00	0.00	0.00	3	13		254,000,000
Maximum		1,200,213	1,162,273	1,232,677	100.00	0.00	0.00	0.00	1.00	35	3,152		569,000,000

SUBJECT F.

B 38	Nov. 13	18,000	22,000	.....	....	...	...	...	...	..	..		396,000,000
B 61	Nov. 26	71,000	140,000	.....	....	...	...	...	...	..	..		430,000,000
B 84	Dec. 12	64,000	90,000	.....	....	...	...	...	...	..	..		360,000,000
B 103	Dec. 28	20,040	27,042	.....	....	...	...	...	...	..	..		459,000,000
B 115	Jan. 14	37,520	41,850	31,880	100.0	0.0	0.0	0.0	0.0	..	..		465,000,000
B 127	Jan. 29	159,860	107,340	132,580	99.3	0.5	0.0	0.2	0.0	..	144	I–II	384,000,000
B 139	Feb. 17	149,700	133,860	115.200	100.0	0.0	0.0	0.0	0.0	..	58	I	469,000,000
B 153	Mar. 6	31,940	30,060	33,140	99.0	0.0	0.0	1.0	0.0	..	12	I	263,000,000
B 165	Mar. 23	59,080	47,410	67,400	100.0	0.0	0.0	0.0	0.0	..	38	I	456,000,000
B 177	April 6	90,240	79,500	.....	Plates lost by melting					14	3,280	I–II	465,000,000
B 189	April 22	53,900	42,200	53,340	100.0	0.0	0.0	0.0	0.0	19	66	III–XXI	498,000,000
B 201	May 7	13,380	12,640	12,040	100.0	0.0	0.0	0.0	0.0	10	21	I–II–XXII	476,000,000
B 213	May 23	63.560	35,980	67,060	100.0	0.0	0.0	0.0	0.0	9	14	I–XXIII	408,000,000
B 225	June 8	1,526,140	1,137,300	380,360	100.0	0.0	0.0	0.0	0.0	40	830	I	477,000,000
B 237	June 25	423,960	429,220	351,380	100.0	0.0	0.0	0.0	0.0	269	2,146	I–VIII	423,000,000
B 249	July 13	89,960	113,320	117,000	100.0	0.0	0.0	0.0	0.0	31	48	I*–III	418,000,000
B 261	July 27	122,500	113,560	158,980	100.0	0.0	0.0	0.0	0.0	6	0		398,000,000
Average		176,164	153,134	125,947	99.86	0.04	0.00	0.10	0.00	50	555		403,000,000
Minimum		13,380	12,640	12,040	99.00	0.00	0.00	0.00	0.00	6	0		263,000,000
Maximum		1,526,140	1,137,300	380,360	100.00	0.50	0.00	1.00	0.00	269	3,280		498,000,000

* Represented by a single colony.

TABLE 8—*Continued.*

SUBJECT G.

Laboratory No.	Date	Unheated Suspension								Spore Material (Heated at 80° C. 15 Minutes)			Microscopic Count
		Aerobic Agar	Anaerobic Agar	Aerobic Gelatin						Aerobic Agar	Anaerobic Blood Agar		
				Total Colonies	Bacteria				Molds		Colonies	Types	
					Non-liq. Acid P. ct.	Non-liq. Alk. P. ct.	Liquefying Acid. P. ct.	Liquefying Alk. P. ct.	P. ct.				
B 42	Nov. 15	73,000	92,000	.....	....	...	...	...	...	..	...		208,000,000
B 65	Nov. 29	1,800	16,000	.....	....	...	...	...	...	..	...		341,000,000
B 88	Dec. 14	1,360	6,440	.....	....	...	...	...	...	..	...		179,000,000
B 107	Jan. 2	9,100	19,500	.....	....	...	...	...	...	..	...		327,000,000
B 119	Jan. 20	19,220	19,940	.....	....	...	...	...	...	..	...		260,000,000
B 131	Feb. 3	5,120	9,840	7,040	98.0	2.0	0.0	0.0	0.0	..	621	I	379,000,000
B 143	Feb. 21	228,358	277,841	241,633	100.0	0.0	0.0	0.0	0.0	..	283	I	534,000,000
B 152	Mar. 4	51,070	40,350	56,827	99.7	0.1	0.1	0.1	0.0	..	2,010	I	480,000,000
B 164	Mar. 19	11,306	11,020	9,720	96.3	3.0	0.7	0.0	0.0	..	262	I–XVIII	439,000,000
B 176	April 3	60,688	46,923	69,424	99.9	0.0	0.0	0.1	0.0	20	1,240	I	474,000,000
B 188	April 20	145,314	106,600	133,309	100.0	0.0	0.0	0.0	0.0	11	1,143	I–II–XVIII	468,000,000
B 200	May 4	27,173	31,865	25,180	99.0	1.0	0.0	0.0	0.0	2	1,483	I–II–IX	446,000,000
B 212	May 20	279,878	165,948	215,078	100.0	0.0	0.0	0.0	0.0	6	1,935	I–II–XVIII	497,000,000
B 224	June 5	100,494	86,072	90,288	100.0	c.0	0.0	0.0	0.0	14	2,909	I	290,000,000
B 236	June 22	259,468	289,575	260,368	100.0	0.0	0.0	0.0	0.0	24	64,398	I–XVIII	416,000,000
B 248	July 9	275,179	202,692	205,090	100.0	0.0	0.0	0.0	0.0	30	319	I	483,000,000
B 264	July 29	170,114	162,327	142,400	90.0	0.0	0.0	0.0	10.0	280	204	I	583,000,000
Average		101,097	93,231	121,363	98.57	0.51	0.07	0.02	0.83	48	6,401		400,000,000
Minimum		1,360	6,440	7,040	90.00	0.00	0.00	0.00	0.00	2	204		179,000,000
Maximum		279,878	289,575	260,368	100.00	3.00	0.70	0.10	10.00	280	64,398		583,000,000

SUBJECT H.

B 48	Nov. 18	13,700	13,000	.....	....	...	.....	...	...	..	...		377,000,000
B 71	Dec. 3	54,000	77,000	.....	....	...	...	...	...	..	...		535,000,000
B 94	Dec. 19	49,400	55,000	.....	....	...	...	...	...	..	...		364,000,000
B 109	Jan. 6	93,800	103,000	94,300	100.0	0.0	0.0	0.0	0.0	..	...		412,000,000
B 121	Jan. 22	111,000	77,500	98,500	100.0	0.0	0.0	0.0	0.0	..	...		383,000,000
B 133	Feb. 6	182,060	182,240	205,800	100.0	0.0	0.0	0.0	0.0	..	...		364,000,000
B 145	Feb. 24	26,680	37,650	49,520	99.9	0.1	0.0	0.0	0.0	..	397	I	451,000,000
B 156	Mar. 10	135,006	146,201	101,777	100.0	0.0	0.0	0.0	0.0	..	441	I–II	517,000,000
B 168	Mar. 25	57,414	47,294	.....	Plates lost		by melting		...	26	140	I–II–XVIII	453,000,000
B 180	April 8	160,383	147,060	200,803	100.0	0.0	0.0	0.0	0.0	24	371	I–III	500,000,000
B 192	April 25	84,505	53,244	58,520	98.0	2.0	0.0	0.0	0.0	10	197	I–XVIII	647,000,000
B 204	May 11	63,994	7,830	49,965	99.9	0.1	0.0	0.0	0.0	17	187	I–III	456,000,000
B 216	May 25	222,495	132,311	221,774	100.0	0.0	0.0	0.0	0.0	..	15	I–XVIII	512,000,000
B 228	June 12	94,750	85,616	100,153	100.0	0.0	0.0	0.0	0.0	72	89	I–III–XVIII	707,000,000
B 240	June 29	551,304	434,267	600,274	100.0	0.0	0.0	0.0	0.0	5	131	I–III–XVIII	536,000,000
B 252	July 15	931,613	141,031	798,867	100.0	0.0	0.0	0.0	0.0	4	19,124	I–II–VIII	322,000,000
B 260	July 24	1,106,737	1,184,358	967,514	100.0	0.0	0.0	0.0	0.0	42	48	I–VIII–XVIII	540,000,000
Average		231,697	172,035	272,905	99.83	0.17	0.00	0.00	0.00	25	1,922		475,000,000
Minimum		13,700	7,830	49,520	98.00	0.00	0.00	0.00	0.00	4	15		322,000,000
Maximum		1,106,737	1,184,358	967,514	100.00	2.00	0.00	0.00	0.00	72	19,124		707,000,000

SUBJECT I.

B 32	Nov. 11	108,000	94,000	.....	....	...	...	...	...	..	..		251,000,000
B 55	Nov. 22	40,000	39,400	.....	....	...	...	...	...	..	...		348,000,000
B 78	Dec. 9	20,000	16,000	.....	....	...	...	...	...	..	...		290,000,000
B 101	Dec. 26	7,650	10,300	.....	....	...	...	...	...	..	...		291,000,000
B 113	Jan. 12	222,800	233,900	344,500	100.0	0.0	0.0	0.0	0.0	..	...		335,000,000
B 125	Jan. 27	19,200	21,740	17,440	99.4	0.0	0.6	0.0	0.0	..	427	I	290,000,000
B 137	Feb. 14	98,700	91,762	106 239	100.0	0.0	0.0	0.0	0.0	..	89	I	498,000,000
B 160	Mar. 14	127,300	107,650	.....	....	...	...	...	...	..	157	I	282,000,000
B 172	Mar. 31	86,360	64,520	98,440	99.7	0.0	0.3	0.0	0.0	4	73	I	442,000,000
B 184	April 13	146,520	106,200	130,960	100.0	0.0	0.0	0.0	0.0	12	378	I	399,000,000
B 196	April 29	38,280	33,040	37,300	99.7	0.3	0.0	0.0	0.0	7	190	I–III–XXIV	457,000,000
B 208	May 15	120,460	102,560	122,360	99.8	0.1	0.1	0.0	0.0	6	226	I–III	478,000,000
B 220	June 1	554,780	402,880	575,540	100.0	0.0	0.0	0.0	0.0	15	786	I–II–VIII	397,000,000
B 232	June 17	260,460	250,980	302,740	100.0	0.0	0.0	0.0	0.0	17	158	I–IX–XXV	492,000,000
B 244	July 3	204,585	195,726	206,601	100.0	0.0	0.0	0.0	0.0	12	108	I–II	612,000,000
B 256	July 20	542,180	561,880	531,020	100.0	0.0	0.0	0.0	0.0	20	13,531	I–II–IX	576,000,000
Average		162,333	145,784	223,921	99.87	0.04	0.09	0.00	0.00	12	1,466		402,000,000
Minimum		7,650	10,300	17,440	99.40	0.00	0.00	0.00	0.00	4	73		251,000,000
Maximum		554,780	561,880	575,540	100.00	0.30	0.60	0.00	0.00	20	13,531		612,000,000

TABLE 8—*Continued.*

SUBJECT J.

Laboratory No.	Date	Unheated Suspensions: Aerobic Agar	Unheated Suspensions: Anaerobic Agar	Unheated Suspensions: Aerobic Gelatin: Total Colonies	Unheated Suspensions: Aerobic Gelatin: Bacteria: Non-liq. Acid P. ct.	Unheated Suspensions: Aerobic Gelatin: Bacteria: Non-liq. Alk. P. ct.	Unheated Suspensions: Aerobic Gelatin: Bacteria: Liquefying Acid. P. ct.	Unheated Suspensions: Aerobic Gelatin: Bacteria: Liquefying Alk. P. ct.	Unheated Suspensions: Aerobic Gelatin: Molds P. ct.	Spore Material (Heated at 80° C. 15 Minutes): Aerobic Agar	Spore Material (Heated at 80° C. 15 Minutes): Anaerobic Blood Agar: Colonies	Spore Material (Heated at 80° C. 15 Minutes): Anaerobic Blood Agar: Types	Microscopic Count
B 31	Nov. 9	2,900	6,687	.....	....	...	...	...	...	..	..		158,000,000
B 54	Nov. 21	2,720	6,360	.....	....	...	...	...	...	..	..		322,000,000
B 77	Dec. 7	62,400	36,500	.....	....	...	...	...	...	..	..		279,000,000
B 100	Dec. 23	6,026	9,640	.....	....	...	...	...	...	..	..		268,000,000
B 111	Jan. 9	Plates	lost	.....	....	...	...	...	...	..	..		298,000,000
B 124	Jan. 25	40,000	41,000	40,000	99.8	0.2	0.0	0.0	0.0	..	..		309,000,000
B 136	Feb. 10	12,000	14,586	11,700	98.0	0.0	0.0	2.0	0.0	..	..		220,000,000
B 148	Feb. 27	22,358	31,182	25,809	99.0	0.0	0.1	0.0	0.0	..	6	I	386,000,000
B 159	Mar. 13	15,813	10,626	.....	Plates lost		by melting		...	..	8	Not determined	364,000,000
B 171	Mar. 30	88,093	86,515	80,395	100.0	0.0	0.0	0.0	0.0	10	0		401,000,000
B 183	April 14	34,420	28,760	17,520	100.0	0.0	0.0	0.0	0.0	8	8	I	439,000,000
B 195	April 29	60,449	31,621	63,700	100.0	0.0	0.0	0.0	0.0	0	13	I	302,000,000
B 207	May 15	2,320	3,240	.....	Plates lost		by melting		...	7	0		383,000,000
B 219	June 1	60,375	46,416	48,540	100.0	0.0	0.0	0.0	0.0	17	2	Not determined	476,000,000
B 231	June 17	31,100	26,680	10,790	99.4	0.3	0.3	0.0	0.0	11	0		368,000,000
B 243	July 4	5,520	4,900	5,600	96.0	0.0	0.0	0.0	4.0	..	10	I	372,000,000
B 255	July 20	67,387	55,300	77,021	99.8	0.0	0.0	0.0	0.2	40	21	XVIII	228,000,000
Average		32,117	27,501	38,108	99.11	0.06	0.05	0.25	0.53	13	7		328,000,000
Minimum		2,320	3,240	5,600	96.00	0.00	0.00	0.00	0.00	0	0		158,000,000
Maximum		88,093	86,515	80,395	100.00	0.30	0.30	2.00	4.00	40	21		476,000,000

SUBJECT K.

B 40	Nov. 14	244,000	212,000	.....	....	...	...	...	...	..	...		313,000,000
B 63	Nov. 27	53,000	65,000	.....	....	...	...	...	...	..	...		259,000,000
B 86	Dec. 13	44,000	49,000	.....	....	...	...	...	...	..	...		232,000,000
B 105	Dec. 30	40,253	56,610	.....	....	...	...	...	...	..	...		227,000,000
B 117	Jan. 16	8 370	15,080	.....	....	...	...	...	...	..	...		264,000,000
B 129	Jan. 31	66,020	80,720	83,660	100.0	0.0	0.0	0.0	0.0	..	519	I	300,000,000
B 141	Feb. 19	38,740	35,800	48,240	100.0	0.0	0.0	0.0	0.0	..	84	I	198,000,000
B 151	Mar. 4	25,173	24,840	15,460	99.7	0.3	0.0	0.0	0.0	..	14	I	385,000,000
B 163	Mar. 19	3,860	5.000	4,220	99.0	0.0	0.0	1.0	0.0	..	25	I	358,000,000
B 175	April 3	34,440	43,520	47,220	100.0	0.0	0.0	0.0	0.0	15	20	I	282,000,000
B 187	April 20	34,180	43,040	33,760	100.0	0.0	0.0	0.0	0.0	10	76	I–III	356,000,000
B 199	May 4	29.040	17,640	17,840	100.0	0.0	0.0	0.0	0.0	2	136	I–II	260,000,000
B 211	May 20	34,820	29,080	28,300	100.0	0.0	0.0	0.0	0.0	8	1,200	I	276,000,000
B 223	June 5	6,480	6,240	6,740	100.0	0.0	0.0	0.0	0.0	14	3,616	I–VIII	317,000,000
B 235	June 23	48,400	52,700	60,640	99.7	0.3	0.0	0.0	0.0	52	2,176	II–VIII	370,000,000
B 247	July 9	67,880	40,840	68,300	99.9	0.1	0.0	0.0	0.0	8	291	II	224,000,000
B 263	July 29	52,860	66,420	57,780	95.0	0.0	0.0	0.0	5.0	181	26,361	I	252,000,000
Average		48,913	49,619	39,347	99.48	0.06	0.00	0.09	0.46	36	2,877		287,000,000
Minimum		3,860	5,000	4,220	95.00	0.00	0.00	0.00	0.00	2	14		198,000,000
Maximum		244,000	212,000	83,660	100.00	0.30	0.00	1.00	5.00	181	26,361		385,000,000

SUBJECT L.

B 51	Nov. 19	163,600	111,500	.....	....	...	...	...	...	..	..		451,000,000
B 74	Dec. 6	3,240	7,700	.....	....	...	...	...	...	..	..		481,000,000
B 97	Dec. 20	9,500	9,500	.....	....	...	...	...	...	..	..		337,000,000
B 108	Jan. 2	12,760	65,700	.....	....	...	...	....	...	..	..		331,000,000
B 120	Jan. 20	10,050	18,020	.....	....	...	...	...	...	..	..		175,000,000
B 132	Feb. 3	33,400	35,000	61,860	100.0	0.0	0.0	0.0	0.0	..	94	I	203,000,000
B 144	Feb. 21	22,180	29,820	24,560	99.8	0.0	0.2	0.0	0.0	..	484	I	370,000,000
B 155	Mar. 9	5,140	5,760	7.520	99.7	0.3	0.0	0.0	0.0	..	295	I	479,000,000
B 167	Mar. 25	39.320	46,040	.....	Plates lost		by melting		...	28	161	I	340,000,000
B 179	April 8	93,560	93,740	110,860	100.0	0.0	0.0	0.0	0.0	18	68	III–IX	390,000,000
B 191	April 24	11,420	11,040	12,900	98.0	2.0	0.0	0.0	0.0	14	53	I–IX	353,000,000
B 203	May 11	22,340	16,860	12,660	98.0	0.0	2.0	0.0	0.0	28	392	I–XXVI	367,000,000
B 215	May 25	306,580	264,840	262,320	100.0	0.0	0.0	0.0	0.0	9	125	I	302,000,000
B 227	June 12	34.260	30,380	31,060	100.0	0.0	0.0	0.0	0.0	50	16	I–III	446,000,000
B 239	June 29	66,040	61,040	60,320	100.0	0.0	0.0	0.0	0.0	9	377	I–XVIII	341,000,000
B 251	July 15	106,720	73,020	147,660	99.8	0.0	0.2	0.0	0.0	12	39,449	I	430.000,000
B 259	July 25	139,940	119,480	167,440	100.0	0.0	0.0	0.0	0.0	60	654	I	507,000,000
Average		63,568	58,896	81,742	99.53	0.23	0.24	0.00	0.00	25	3,514		371,000,000
Minimum		3,240	5,760	7,520	98.00	0.00	0.00	0.00	0.00	9	16		175,000,000
Maximum		306,580	264,840	262,320	100.00	2.00	2.00	0.00	0.00	60	39,449		507,000,000

technic have been pointed out by Stern,[14] who has shown that the former's conclusions are not justified. From the results given in Tables 8 and 9, it is evident that the number of cultivable facultative anaerobes in the feces is not a reliable indication of the extent of bacterial multiplication in the digestive tract. This question will be more fully discussed below.

From the fecal suspensions heated to 80° C. for 15 minutes, two sets of plates were made, for aerobic spores and for anaerobic spores. Ordinarily quantities of 0.25 and 0.50 c.c. of Suspension No. 2, Spores, were plated in litmus lactose agar aerobically. Ninety-seven such examinations for aerobic spores are tabulated. Bacteria of this class were nearly always present in sufficient numbers to appear on these plates (0.5 c.c. of Suspension No. 2 represents 0.0005 gm. feces). Thin spreading colonies with alkaline reaction, characteristic in appearance, were observed 94 times in the 97 examinations. It is probable that this species is a normal component of the human intestinal flora, and for this reason it has been subjected to more detailed studies* which have shown it to be a member of the Urobacillus group. The number of aerobic spores varied from 3,556 per milligram in Subject A, July 31, to 0 in Subject J, Apr. 29, the only examination in which growth was entirely absent from these plates. The average of the 97 examinations was 65 aerobic spores per milligram feces.

Anaerobic plates† inoculated with similar quantities of Suspension No. 2, Spores, were made on blood agar in 139 different stools of this series. The medium was prepared by adding about 1 c.c. of naturally sterile, defibrinated blood (of rabbit, dog, cat, or goat according to availability) to the tube of plain agar, melted and cooled to 45° C., immediately before pouring. The plates were incubated 48 hours under anaerobic conditions. An attempt was made to isolate as many different species as possible from these plates and to preserve them for further study. In the tables 26 types are indicated. Of these only six deserve serious consideration as the others are represented by a single occurrence only, except Type XV which

* The work upon identification of the bacteria isolated from feces will appear in a subsequent communication.

† We owe this method to the suggestion of Professor Theobald Smith, who has found it well adapted to the isolation of *B. welchii* from feces.

occurred twice in the same individual. The types of sporogenic anaerobes which were repeatedly isolated were Numbers I, II, III, VIII, IX, and XVIII. Type I is undoubtedly the species described by Welch and Nuttall[20] under the name, *B. aerogenes capsulatus*. Type II resembles it very closely but forms spores more readily. Both of these hemolyze blood, producing clear areas around their colonies in the blood agar plates. Type III does not hemolyze the blood, but produces a green coloration about the colony on the blood agar plates. Type VIII appears to be related to *B. edematis* but is nonpathogenic to laboratory animals. In milk fermentation tubes it rapidly digests the casein without the formation of acid. Type IX resembles Type VIII very closely, being distinguished from it chiefly by a slower liquefaction of gelatin. Type XVIII is morpohlogically distinct from the others as it forms a large oval terminal spore much wider than the cell. In this it resembles Bienstock's[2] *B. putrificus*, the Köpfchen bacillus described by Escherich,[5] and the drum-stick bacillus described by Tavel.[16] It is quite probable that Types I and II are merely varieties of the same species, and that Types VIII and IX are also similarly related. If this should be the case there would be left for consideration only four species. In the examination of 139 different stools for sporogenic anaerobes according to this method, Type I was recognized in 113, Type II in 25, Type III in 21, Type VIII in 14, Type IX in 8, and Type XVIII in 12. The more detailed description of these forms must be left for a subsequent communication.

The number of colonies appearing upon these anaerobic spore plates was exceedingly variable. The greatest number, 64,398 cultivable anaerobic spores per milligram feces, occurred in Subject G, June 22, consisting almost wholly of Type I. In nine subjects, anaerobic spore organisms were found at every examination. Three individuals, Subject D, Feb. 27, Apr. 10, and July 1, Subject F, July 27, and Subject J, Mar. 30, May 15, and June 17, failed to yield cultures of sporogenic anaerobes by this method, upon the dates named, altho they were found at other examinations. The average of all the 139 examinations was 1,790 cultivable anaerobic spores per milligram fresh feces. It should be kept in mind that these figures represent spores, and not the total number of sporogenic anaerobes.

Table 9 summarizes the figures given in Table 8, together with the results of the microscopic count of the fecal bacteria of Table 2.

TABLE 9.

Bacteria per Milligram Fresh Feces.

Summary of Colony Counts and of Direct Microscopic Counts.

Subject		Aerobic Agar	Anaerobic Agar	Aerobic Gelatin	Acid non-liq. P. ct.	Aerobic Spores	Anaerobic Spores	Microscopic Count
A.....	Maximum	94,691	57,642	90,860	100.00	3,556	13,698	539,000,000
	Mean	**35,632**	**31,583**	**35,421**	**99.30**	**411**	**3,595**	**394,000,000**
	Minimum	7,100	10,000	6,400	97.00	4	97	244,000,000
B.....	Maximum	747,000	658,160	736,440	100.00	51	964	425,000,000
	Mean	**133,856**	**108,443**	**127,415**	**99.04**	**20**	**155**	**301,000,000**
	Minimum	14,960	9,840	9,160	92.00	7	6	151 000,000
C.....	Maximum	681,728	680,737	760,604	100.00	538	449	678,000,000
	Mean	**103,645**	**112,947**	**165,927**	**98.21**	**100**	**83**	**461,000,000**
	Minimum	1,787	4,160	7,780	83.00	6	4	341,000,000
D.....	Maximum	74,000	61,920	87,135	100.00	121	3,538	448,000,000
	Mean	**26,637**	**24,699**	**27,950**	**99.67**	**23**	**485**	**305,000,000**
	Minimum	2,420	2,900	3,140	98.00	3	0	193,000,000
E.....	Maximum	1,200,213	1,162,273	1,232,677	100.00	35	3,152	569,000,000
	Mean	**279,210**	**268,963**	**280,917**	**99.92**	**18**	**422**	**424,000,000**
	Minimum	30,600	36,000	41,500	99.00	3	13	254,000,000
F.....	Maximum	1,526,140	1,137,300	380,360	100.00	269	3,280	498,000,000
	Mean	**176,164**	**153,134**	**125,947**	**99.86**	**50**	**555**	**403,000,000**
	Minimum	13,380	12,640	12,040	99.00	6	0	263,000,000
G.....	Maximum	279,878	289,575	260,368	100.00	280	64 398	583,000,000
	Mean	**101,097**	**93,231**	**121,363**	**98.57**	**48**	**6,401**	**400,000,000**
	Minimum	1,360	6,440	7,040	90.00	2	204	179,000,000
H.....	Maximum	1,106,737	1,184,358	967,514	100.00	72	19,124	707,000 000
	Mean	**231,697**	**172,035**	**272,905**	**99.83**	**25**	**1 922**	**475,000,000**
	Minimum	13,700	7,830	49,520	98.00	4	15	322,000,000
I.....	Maximum	554,780	561,880	575,540	100.00	20	13,531	612,000,000
	Mean	**162,333**	**145,784**	**223,921**	**99.87**	**12**	**1,466**	**402,000,000**
	Minimum	7,650	10,300	17,440	99.40	4	73	251,000,000
J.....	Maximum	88,093	86,515	80,395	100.00	40	21	476,000,000
	Mean	**32,117**	**27,501**	**38,108**	**99.11**	**13**	**7**	**328,000,000**
	Minimum	2,320	3,240	5,600	96.00	0	0	158,000,000
K.....	Maximum	244,000	212,000	83,660	100.00	181	26,361	385,000,000
	Mean	**48,913**	**49,619**	**39,347**	**98.48**	**36**	**2,877**	**287,000,000**
	Minimum	3,860	5,000	4,220	95.00	2	14	198,000,000
L.....	Maximum	306,580	264,840	262,320	100.00	60	39,449	507,000,000
	Mean	**63,568**	**58,896**	**81,742**	**99.53**	**25**	**3,514**	**371,000,000**
	Minimum	3,240	5,760	7,520	98.00	9	16	175,000,000
All subjects	Maximum	1,526,140	1,184,358	1,232,677	100.00	3,556	64,398	707,000,000
	Mean	**116,239**	**103,903**	**128,414**	**99.38**	**65**	**1,790**	**379,000,000**
	Minimum	1,360	2,900	3,140	83.00	0	0	151,000,000

It is clearly evident that the number of bacteria brought to development by our culture methods is very small in comparison with the result of the direct counts, on the average about one germ in three thousand appearing as a colony upon the plates.

RELATION BETWEEN CULTIVABLE AND VISIBLE FECAL BACTERIA.

That only a small fraction of the bacteria of the human feces appears in cultures has been well known for some time (see Schmidt und Strasburger,[12] pp. 281–86). Klein[10] considers the bacteria which fail to develop to be dead and their death to be the result of specific germicidal action of the intestine and its secretions. From examinations of the intestinal flora of the rabbit he concluded that the bacteria did not multiply at any part of the digestive tract, but were being killed at every point and being disintegrated, especially in the large intestine. Klein's deduction from his experiments meets a serious difficulty in the well known fact that enormous numbers of bacteria continue to be excreted in the feces even when excluded from the food. Belonowski[1] has fed mice sterilized food exclusively for seven months without any appreciable diminution in the excretion of cultivable fecal bacteria. Moreover, the germicidal action of the digestive juices, with the exception of the gastric juice, has not been demonstrated. In fact, the bile, the pancreatic juice, and the enteric secretion are all fairly good culture media *in vitro*. The first effect of a germicide upon bacteria is to inhibit growth and multiplication, and the enormous number of bacterial cells in the feces shows that the conditions for bacterial growth are exceptionally favorable in the intestinal tract, rather than inhibitive, not to mention germicidal.

We may better understand the biology of intestinal bacteria if we keep clearly in mind the nature of bacterial growth and death, in general, as it has been observed in the culture tube and upon dead matter. In common with other living cells, bacteria utilize food materials, thus tending to exhaust the available supply, and they produce and excrete various substances, some of which are very unfavorable to their own growth and existence. It may be said that, in general, living cells are able to endure deprivation of food for a much longer period than they can endure the presence of the waste products of their own metabolism. That the products of bacterial growth exert a pronounced germicidal effect upon their own species is already well established as a general principle.* Hehewerth[7] has shown this to be the case for *B. coli*, in cultures of which the bacterial cells begin to perish even before multiplication has ceased. In agar

* Gotschlich in Kolle und Wassermann,[11] *Handbuch*, I, p. 116.

flask cultures grown at 37° C. for five days and still perfectly moist, we have found the number of living bacteria estimated by the plate method to be, in one experiment, $\frac{1}{15}$ of the total cells counted microscopically; in another experiment $\frac{1}{8}$ of the total visible cells. In agar flask cultures incubated eight days at 37° and still perfectly moist, only $\frac{1}{40}$ of the cells counted microscopically were found capable of development on agar plates. Each one of these visible cells was at the moment of its origin essentially capable of independent proliferation, given favorable medium and environment. Its death necessitates the assumption of definite unfavorable influences present in the culture. This condition of a culture has long been spoken of as exhaustion of the medium, after the conception of Pasteur that the nutritive substances were entirely used up and the bacterial cells starved to death, but in reality the exhaustion of the medium is due in a much larger measure to the accumulation of waste products. This fact will be made clearly evident by a consideration of the natural limitation of bacterial growth in cultures.

Eijkman[4] has shown that when gelatin is inoculated with 6,000,000 living cells of *B. coli* per milligram of medium, no growth takes place, only gradual death and disintegration of the bacteria, and he has clearly demonstrated that bacterial growth in general is limited not by the exhaustion of the nutritive substances of the medium but by the accumulation of very definite *inhibitory* metabolic products of the bacteria, some of which at least are diffusible, thermolabile, and not appreciably filterable through porcelain. He was able to regenerate an "exhausted" medium by filtration through porcelain or by heating, in neither of which procedures is any nutritive substance added, and yet the resulting material again supported bacterial growth. He has also shown that these same inhibitory substances are present in human feces. These results have been confirmed by Conradi and Kurpjuweit,[3] who were able to detect this inhibitory substance in cultures of *B. coli* after the first hour of growth at 37° C., and in human feces even when diluted 10,000 times. They suggest the name "Autotoxine" for this class of substances. These experiments merely bring more clearly and more definitely before us what has been evident in a vague sort of a way to anyone who has made plates or transplanted stock cultures.

Besides this unfavorable effect upon their own species, bacterial products also frequently exert an inhibitory and germicidal action upon other species, and this hetero-antagonism has been especially studied in the case of *B. coli* by Eijkman,[4] and by Conradi and Kurpjuweit.[3] It seems evident, therefore, that Escherich[5] was correct in ascribing to the concentration of intestinal contents by absorption of water and the consequent accumulation of bacterial waste products, an important rôle in restricting, weakening, and destroying the intestinal bacteria, and it appears safe to assume that a large part of the great mass of fecal bacteria which fail to grow are actually dead cells, killed not by the action of the body cells, but largely by the same causes which kill bacteria in artificial cultures, their own metabolic products.

Some of those individual cells of readily cultivable species which fail to develop may not be dead but only weakened so that they do not grow upon solid media, altho they may develop in broth or other more favorable media. Where bacteria are dying there are some almost but not quite dead. This conception serves to account for the discrepancies between quantitative estimations of the same species on different media, as has been pointed out by Escherich.[5]

The remarkable variations in the number of cultivable *B. coli* in the feces of the same individual are best accounted for, not by differences in the amount of multiplication of this species in the intestine but by the differences in the extent to which the bacterial cells have died before passage of the feces. From the researches of Escherich[5] and of Tissier[18] upon nurslings, of Tissier[19] upon older children, and of Gessner[6] upon adults, it is evident that bacteria of the *B. coli* group (including *B. lactis aerogenes*, Escherich) are the chief bacteria of the small intestine and reach their highest development in the cecum and upper colon, thus belonging to the flora of the upper part of the intestine rather than the lower. This group would, therefore, be especially subject to unfavorable influences in the lower colon, specific antagonistic substances resulting from its own excessive proliferation at higher levels, the drying of the intestinal contents with consequent crowding of bacterial cells, the absorption and exhaustion of nutritive material, and possibly the antagonistic action of other bacterial species better adapted to the conditions of the

lower part of the intestine. This last mentioned factor has been shown to be of particular importance in breast fed infants by Tissier.[18]

In general the colon bacilli flourish where the digestion of proteins and sugars is in progress, in the presence of abundant intestinal juice, and they perish as absorption is being completed. Other things being equal, the survival of a large number of *B. coli* in the feces would indicate that these conditions favorable to it had persisted until the moment of excretion from the body was more nearly approached than is ordinarily the case, due either to increased rapidity of peristalsis in the large intestine, or to incomplete or delayed absorption of water, protein, and possibly of carbohydrate. Those stools which gave rise to less than 10,000 colonies of *B. coli* per milligram feces and those which gave rise to more than 200,000 colonies per milligram have been selected for comparison especially in regard to the clinical examinations of the feces. The clinical examinations of the feces were made for the most part by Dr. W. S. Chapin, clinical assistant in the laboratory. In the tables, (+) indicates a very small amount or trace; +, a small amount; ++, a moderate amount; +++, considerable; ++++, a large, and +++++, a very large quantity. The examinations for striated muscle, starch, granulose bacteria, and fat were made with the aid of the microscope. Table 10 presents the data for the 29 stools with less than 10,000 bacterial colonies per milligram. None of these stools showed mucous flakes, and only three, Subject G, Jan. 2, Subject K, Jan. 16 and Mar. 19, showed any excess of striated muscle fibers. In all the others the low colony count is associated with a fairly complete utilization of the muscle element of the food. In two of the exceptional cases, Subject K, Stools No. B 117 and No. B 163, there was undoubtedly marked fermentation due to anaerobes as indicated by the odor, abundance of gas bubbles in the feces, the greater number of colonies developed under anaerobic conditions, and the predominance of positive bacilli in the fermentation tube sediments. In the other exception, Subject G, Jan. 2, Stool No. B 107, the evidence is not so conclusive, but the anaerobic plates yielded 19,000 colonies per milligram and the glucose fermentation tube became almost filled with gas, 9.7 cm.

TABLE 10.

CLINICAL DATA UPON THE STOOLS SHOWING LESS THAN 10,000 CULTIVABLE *B. coli* PER MILLIGRAM FECES.

Subject	Lab. No.	Date	Colonies per mg.	Consistency	Dry Substance P. ct.	Odor	Gas Bubbles	Mucous Flakes	Striated Muscle	Starch	Granulose Bacteria	Fat
A.....	B 60	Nov. 26	7,100	Formed	....	Strong	o	o	+	+	+	+
B.....	B 246	July 6	9,800	Formed	25.2	Normal	o	o	o	o	o	++++
C.....	B 39	Nov. 13	1,787	Formed	....	Normal	o	o	o	o	+	+
	B 62	Nov. 26	4,780	Formed	....	Normal	o	o	(+)	+	o	o
	B 154	Mar. 6	7,400	Mushy	20.1	.....	o	o	+	+++	(+)	++
	B 166	Mar. 23	3,160	Formed	30.4	Normal	o	o	+	o	o	o
	B 190	April 22	9,100	Formed	24.3	Very strong	o	o	o	+	o	o
D.....	B 99	Dec. 23	8,007	Formed	....	Strong	o	o	+	(+)	(+)	++++
	B 135	Feb. 10	7,620	Formed	20.5	Sweetish	+++	o	o	+++	(+)	(+)
	B 157	Mar. 11	6,080	Formed	25.0	Normal	o	o	+	+	(+)	(+)
	B 193	Mar. 27	2,420	Mushy	20.2	Sweetish	+++	o	+	+++	+++	+
G.....	B 65	Nov. 29	1,800	Formed	....	Normal	o	o	+	o	o	o
	B 88	Dec. 14	1,360	Formed	....	Normal	o	o	+	+++	o	o
	B 107	Jan. 2	9,100	Formed	....	Normal	o	o	+++	+++	+	o
	B 131	Feb. 3	5,120	Formed	30.5	Normal	o	o	+	+	o	+
	B 164	Mar. 19	9,720	Formed	29.2	Normal	o	o	o	+	+++	++++
H.....	B 204	May 11	7,830	Formed	31.1	Strong	o	o	o	o	o	o
I.....	B 101	Dec. 26	7,650	Formed	....	Normal	o	o	o	++	o	o
J.....	B 31	Nov. 9	2,900	Soft	....	Normal	o	o	+	o	+	+
	B 54	Nov. 21	2,720	Soft	....	Strong	o	o	(+)	o	+	+
	B 100	Dec. 23	6,026	Formed	....	Sweetish	+	o	+	+++	+	++++
	B 207	May 15	2,320	Formed	22.4	Sweetish	o	o	o	(+)	+	o
	B 243	July 4	4,900	Mushy	21.4	Normal	o	o	o	+	+++	++
K.....	B 117	Jan. 16	8,370	Mushy	....	Offensive	+++	o	+++	+++	+++	o
	B 163	Mar. 19	3,860	Formed	18.7	Putrefactive	+++	o	++	+++	++++	++++
	B 223	June 5	6,240	Formed	24.5	Normal	o	o	+	o	o	(+)
L.....	B 74	Dec. 6	3,240	Soft	....	Normal	o	o	o	+	+	+
	B 97	Dec. 20	9,500	Formed	....	Normal	o	o	+	++++	++++	o
	B 155	Mar. 9	5,140	Formed	38.6	Strong	o	o	o	o	o	o

In Table 11, similar data are given for the 32 stools yielding more than 200,000 *B. coli* colonies per milligram. In 12 of these, mucous flakes were observed and the 3 counts above a million per milligram were all associated with the presence of excessive mucus. In 15 of the 32, striated muscle was present in increased quantity. In 6, the dry substance of the stool was less than 20 per cent. There are 6 of the 32 stools in which no excess of mucus, muscle residue, or water was noted. A comparison of these two tables suggests a definite relation between these signs of incomplete absorption, increased intestinal secretion, and accelerated peristalsis on the one hand, and a large number of cultivable *B. coli* in the feces on the other, but it is also clear that other factors enter into and confuse this relation. The extent of multiplication of *B. coli* in the intestine seems to have very little relation to variations in the number of cultivable *B. coli* in the feces, this latter depending upon factors which affect the extent of dying of the bacterial cells.

The assumption that all the visible bacteria which fail to develop on plate cultures are dead is, however, not justifiable. Special methods of culture may be expected to bring to development new species of fecal bacteria. Already several kinds of bacteria requiring special methods for their isolation have been shown to be fairly constant inhabitants of the intestine, of which the acidophile or acid resisting group and the sporogenic anaerobes may be mentioned. Besides these, there are certain forms such as the slender spirochetes which are visibly alive in the feces, and yet have not been brought to development by culture methods. It is not improbable that some species, cultivable with the greatest difficulty, may be very important members of the fecal flora, as their adaptation to such a strictly parasitic existence would suggest. To estimate the quantity of the living fecal bacteria not cultivable by our present methods, is manifestly impossible, and until we possess some better criterion by which to recognize living and dead bacteria, the results of culture experiments must be interpreted with caution. One must always keep in mind the possibility that some of the species under consideration may not be cultivable by the methods employed.

TABLE II.

CLINICAL DATA UPON THE STOOLS SHOWING MORE THAN 200,000 CULTIVABLE *B. coli* PER MILLIGRAM FECES.

Subject	Lab. No.	Date	Colonies per mg.	Consistency	Dry Substance P. ct.	Odor	Gas Bubbles	Mucous Flakes	Striated Muscle	Starch	Granulose Bacteria	Fat
B.....	B 56	Nov. 22	229,000	Mushy	....	Strong	o	o	++	o	+++	+
	B 79	Dec. 9	324,000	Mushy	....	Strong	+++	o	(+)	+	o	+
	B 150	Mar. 2	747,000	Mushy	19.0	Strong	o	o	+++	o	++++	o
C.....	B 140	Feb. 20	359,924	Formed	17.3 (?)	Putrefactive	o	+++	o	(+)	+	++
	B 238	June 25	681,728	Formed	31.3	Normal	o	o	+++	o	o	+
	B 262	July 27	223,840	Formed	23.3	Strong	o	o	+++	++	++++	+++
E.....	B 118	Jan. 16	231,000	Formed	....	Sweetish	o	++	+	+	o	o
	B 142	Feb. 19	221,833	Formed	23.1	Normal	o	o	++	o	+	+
	B 149	Mar. 2	1,200,213	Formed	27.3	.....	o	+++	+	o	o	o
	B 161	Mar. 16	449,231	Soft	19.0	Normal	o	o	(+)	+++	++++	o
	B 185	April 16	290,920	Formed	28.6	Normal	o	(+)	(+)	+++	o	++
	B 221	June 3	833,561	Mushy	23.2	Sweetish	o	o	o	o	+	(+)
	B 233	June 19	232,271	Formed	28.1	Putrefactive	++	o	++	o	+++	+
	B 257	July 22	299,074	Formed	28.8	Strong	o	o	o	o	+	++
F.....	B 225	June 8	1,526,140	Formed	28.6	Strong	o	++	(+)	o	o	++++
	B 237	June 25	423,960	Formed	30.4	Strong	o	o	(+)	o	++++	++
G.....	B 143	Feb. 21	228,358	Formed	29.9	.....	o	++	o	o	o	(+)
	B 212	May 20	279,878	Formed	29.9	Normal	o	o	o	o	++++	+
	B 236	June 22	259,468	Mushy	19.5	Putrefactive	+++	o	+++	o	o	+++
	B 248	July 9	275,179	Formed	31.2	Sweetish	o	o	+	+	+	o
H.....	B 180	April 8	200,803	Formed	32.4	Strong	o	+	o	(+)	o	o
	B 216	May 25	222 495	Formed	27.0	Putrefactive	o	o	++++	o	+	+++
	B 240	June 29	551,304	Formed	23.9	Strong	o	o	+	o	o	o
	B 252	July 15	931,613	Part fluid	16.9	Putrefactive	o	o	+++++	o	o	++++
	B 260	July 24	1,106,737	Formed	31.1	Strong	o	++	+++	o	o	+++
I.....	B 113	Jan. 12	222,800	Formed	....	.....	o	++	++	+	o	o
	B 220	June 1	554,780	Formed	25.1	.....	o	++	o	o	o	o
	B 232	June 17	260,460	Formed	28.1	Strong	o	+++	++++	o	o	+
	B 244	July 3	204,585	Formed	27.2	Normal	o	o	+	o	o	++
	B 256	July 20	542,180	Formed	27.0	Strong	o	++++	++	+	+	+++
K.....	B 40	Nov. 14	244,000	Soft	....	Normal	o	o	++	....	....	+
L.....	B 215	May 25	306,580	Soft	18.2	Strong	+++	o	++	+++	+++	o

FERMENTATION TUBES.

Fermentation tubes of sugar broth were inoculated with the mixt fecal flora of each stool examined. The medium was prepared from fresh meat, fermented with *B. coli* according to Smith's method. To the resulting nearly sugar-free broth, two per cent of the particular sugar was added, the medium filled into fermentation tubes (without foot) and sterilized in the autoclave. Pure dextrose, levulose, lactose, and saccharose were the sugars used. Each tube was inoculated with 0.25 c.c. of Suspension No. 1 (equivalent to 0.0025 gm. feces). The length of the closed arm was very nearly 12.5 cm. in each case. The quantity of gas produced has been recorded in linear centimeters, and the amount of this gas absorbed by alkali as percentage of the total gas.

The results of these fermentation tube inoculations are recorded in Table 12. The amount of gas produced showed considerable variation. Thus in the dextrose broth, Subject A, Jan. 6, the entire closed arm was filled with gas, while in Subject L, Mar. 9, only 0.9 cm. was produced. Of the twelve individuals, Subject A showed the largest average amount of gas in the dextrose broth, 7.3 cm.; Subject F, the smallest, 3.9 cm. The average for the 12 subjects was 5.2 cm. gas in the dextrose broth. Similar variations in the amount of gas produced also occurred in the other kinds of sugar broth, as will be seen upon examination of Table 12 or of the summary table. It is of interest to note that on the average the lactose broth excelled in the amount of gas produced, and this is also true of the average figures of every one of the 12 individuals (see Table 13).

The portion of the gas absorbable by alkali varied as a rule from $\frac{1}{5}$ to $\frac{2}{5}$ of the total, corresponding roughly to the gas formula of the *B. coli* group.

From the results of plate cultures one might expect a predominant growth of *B. coli* in these fermentation tubes. Microscopic examination of the sediments in the tubes after 24 hours' incubation, however, always revealed a considerable variety of bacteria, gram-positive and gram-negative bacilli and cocci being constantly observed. The predominant type of organism in the gram-stained sediments*

* Differences in the character of the sediments of the different sugar broth tubes inoculated from the same stool were frequently noted.

is indicated in the table. Gram-positive rods were predominant in six instances, in Subjects A, H, and K. In four of these, in Subjects A and K, the closed arm of the fermentation tube was nearly or quite filled with gas. In the other two, in Subject H, the tubes were about half full of gas. The gram-positive bacilli associated with this marked abundance of gas were probably *B. welchii*. On the other hand, there were several instances of abundant gas production in which gram-negative bacilli were predominant in the sediments. No branched forms were ever observed. The interpretation of results of this character presents great difficulties. It will be necessary to study more fully the character and action of each of the various species present in these sediments before a rational theory applicable to their associated action can be formulated. So far, one fact is certain—the growth in these fermentation tubes inoculated with 2.5 mg. of normal human feces is not merely a culture of *B. coli* but a number of species are here brought to development, which fail to appear on the agar plates.

One object in undertaking these fermentation tests was to test the clinical significance of variation in the amount and composition of the gas produced. The results show very marked variation in the amount of gas even in healthy men, and it is evident that the cause* of these variations is not simple.

At present, work is in progress to isolate the various species from these fermentation tubes and determine their identity and action upon the sugars in pure and in mixt cultures. So far as we have been able to discover, the gas production is due to the activity of bacteria belonging either to the *B. welchii* group or the *B. coli* group. These bacteria were present in practically all the stools examined in sufficient quantity, as shown by plate examinations, so that each fermentation tube inoculated with 0.0025 gm. feces would contain

* Herter, to whose work[8] we owe this application of the fermentation tube, apparently considers these variations to be an expression and a measure of the alterations in the gas forming function of the aerogenic species occurring in the intestine, altho his recent statements[9] are not quite clearly specific on this point: "*Observations were made twice weekly on the gas production of the mixed fecal flora in dextrose-bouillon fermentation tubes in the hope of detecting any influence that might possibly be exerted by the sodium benzoate on the gas forming function of the intestinal bacteria*" (Benzoate report, p. 579). "*It is thus clear that large doses of sodium benzoate strongly tend to depress the ability of the fecal bacteria to form gas. The explanation of this fact is not at present clear. The depression in gas formation is certainly not due to the presence of sodium benzoate in the feces, since it was not possible to recover benzoic acid in amounts sufficient to cause such an effect. But it may be due to some action of the benzoate on the bacteria of the digestive tract at higher levels than the colon, or to an action on the digestive juices*" (Benzoate report, p. 605).

TABLE 12.

FERMENTATION TUBES.

SUBJECT A.

Laboratory No.	Date	Inoculated with Mixed Fecal Flora Unheated												Inoculated with Spore Material, Heated at 80° C. for 15 Minutes					
		Sugar Broth									Litmus Milk				Litmus Milk				Blood Broth	
		Dextrose		Levulose		Lactose		Saccharose		Predominant Bacteria in Gram-stained Sediments	Gas Cms.	Coagulation	Digestion	Reaction	Gas Cms.	Coagulation	Digestion	Reaction	Gas Cms.	Capsules
		Gas Cms.	CO_2 %	Gas Cms.	CO_2 %	Gas Cms.	CO_2 %	Gas Cms.	CO_2 %											
B 37	Nov. 12	5.0	40	2.9	24	3.2	28	....	..	.....	....	....	....	....	....	....	....	....	....	....
B 60	Nov. 26	6.0	33	3.4	24	8.9	39	6.8	41	Negative rods	....	....	....	....	....	....	....	....	....	....
B 83	Dec. 10	5.0	32	3.7	30	3.4	32	1.2	17	Negative rods	....	....	....	....	....	....	....	....	....	....
B 110	Jan. 6	12.8	37	2.5	16	10.2	29	9.4	37	.....	....	....	....	....	....	....	....	....	....	....
B 122	Jan. 22	6.7	34	6.9	36	6.5	32	7.0	42	Negative rods	Slt.	+	o	Acid	....	....	....	....	....	....
B 134	Feb. 6	7.2	34	6.3	29	8.3	36	2.2	19	Negative rods	Slt.	+	o	Acid	....	....	....	....	....	....
B 146	Feb. 24	12.4	32	10.5	..	13.3	30	11.4	30	Positive rods	....	....	....	....	....	....	....	....	Full	....
B 158	Mar. 11	8.7	33	7.0	34	10.5	..	3.8	28	Mixed	....	....	....	....	....	....	....	....	Full	+
B 170	Mar. 27	7.0	38	6.1	39	7.4	38	0.4	..	Negative rods	....	....	....	....	....	....	....	....	Full	+ +
B 182	April 10	6.5	35	5.7	37	5.6	23	6.1	37	Negative rods	....	....	....	....	....	....	....	....	0.2	+ o
B 194	April 27	5.9	29	5.5	29	6.5	32	3.0	30	Mixed	....	....	....	....	....	....	....	....	Full	....
B 206	May 13	4.8	31	3.4	24	5.0	28	1.9	21	Negative rods	Slt.	+	o	Acid	Full	+	o	Acid	Full	+ +
B 218	May 28	8.7	32	6.3	30	9.3	35	4.0	25	Mixed	Full	+	o	Red.	Full	+	o	Acid	Full	+ +
B 230	June 15	7.1	26	6.2	24	9.4	18	9.6	14	Negative rods	8.2	+	o	Acid	Full	Par.	o	Acid	Full	+ +
B 242	July 1	5.2	29	7.5	34	9.7	41	5.2	33	Mixed	7.5	+	o	Acid	0.0	0.0	+ + +	Alk.	2.5	o +
B 254	July 17	6.5	38	6.3	28	7.9	32	4.4	31	Mixed	8.2	+	o	Red.	10.2	+	o	Acid	Full	+ +
B 266	July 31	8.9	30	4.2	30	7.7	32	6.4	34	Negative rods	Full	+	o	Acid	Full	+	+	....	Full	
Average		7.3	33	5.5	29	7.8	32	5.2	29											
Minimum		4.8	26	2.5	16	3.2	18	0.4	14											
Maximum		12.8	40	10.5	39	13.3	41	11.4	41											

SUBJECT B.

B 33	Nov. 10	3.7	35	4.9	33	5.3	30	3.1	29	.....	....	....	....	....	....	....	....	....	....	....
B 56	Nov. 22	5.0	32	3.9	41	6.1	34	5.5	44	.....	....	....	....	....	....	....	....	....	....	....
B 79	Dec. 9	3.5	26	4.0	30	7.2	30	2.0	20	.....	....	....	....	....	....	....	....	....	....	....
B 102	Dec. 26	2.0	20	2.8	22	4.9	12	3.0	40	Negative rods	....	....	....	....	....	....	....	....	....	....
B 114	Jan. 12	5.6	26	4.0	12	8.4	34	11.5	..	.....	3.4	+	o	Acid	....	....	....	....	....	....
B 126	Jan. 27	5.0	24	5.0	24	8.5	31	4.9	26	.....	4.5	Firm	o	Acid	....	....	....	....	....	....
B 138	Feb. 13	6.0	31	5.4	33	7.7	29	6.1	37	Negative rods	3.5	+	o	Acid	....	....	....	....	....	....
B 150	Mar. 2	2.6	23	1.3	23	3.3	27	2.8	21	Positive cocci	....	....	....	....	....	....	....	....	10.7	....
B 162	Mar. 16	2.2	22	....	..	4.7	21	1.4	28	Negative cocci	....	....	....	....	....	....	....	....	Full	....
B 174	April 1	4.5	33	3.5	28	7.3	39	5.1	37	Positive cocci	....	....	....	....	....	....	....	....	Full	....
B 186	April 16	3.4	29	5.2	32	6.0	36	7.1	38	Mixed	....	....	....	....	....	....	....	....	Full	....
B 198	May 1	3.7	29	2.7	25	6.9	39	4.2	33	Positive cocci	....	....	....	....	....	....	....	....	Full	....
B 210	May 18	4.2	28	4.3	27	4.7	27	4.2	26	Positive cocci	0.2	+	o	Acid	0.0	Un	chang	ed	0.0	No. gr'th
B 222	June 3	5.4	31	3.5	25	7.6	34	3.3	30	Negative rods	4.5	+	o	Acid	Full	+	o	....	Full	+
B 234	June 19	6.8	30	5.0	32	7.5	32	4.7	29	Positive cocci	3.5	+	o	Acid	Full	+	o	Acid	Full	+
B 246	July 6	6.8	30	5.7	26	9.3	30	3.1	25	Positive cocci	Full	+	o	Acid	Full	+	+	Acid	Full	+
B 258	July 22	5.0	38	3.7	32	5.3	33	5.9	39	Mixed	4.0	+	o	Acid	Full	+	o	Acid	Full	+
Average		4.4	27	4.1	28	6.5	30	4.6	31											
Minimum		2.0	20	1.3	12	3.3	12	1.4	20											
Maximum		6.8	38	5.7	41	9.3	39	11.5	44											

SUBJECT C.

B 39	Nov. 13	3.3	33	2.2	27	5.2	31	3.7	30	.....	....	....	....	....	....	....	....	....	....	....
B 62	Nov. 26	3.9	28	2.9	24	3.2	17	5.0	32	Mixed	....	....	....	....	....	....	....	....	....	....
B 85	Dec. 12	6.7	28	1.7	18	8.1	31	2.8	25	.....	....	....	....	....	....	....	....	....	....	....
B 104	Dec. 28	3.7	33	3.5	26	6.3	39	2.6	39	Negative rods	+	+	o	Acid	....	....	....	....	....	....
B 116	Jan. 14	7.1	31	6.4	35	9.1	38	11.2	27	Negative rods	+	+	o	Acid	....	....	....	....	....	....
B 128	Jan. 29	6.8	30	7.2	33	8.2	36	4.8	37	Negative rods	+	+	o	Acid	....	....	....	....	....	....
B 140	Feb. 20	7.0	30	5.2	29	7.9	30	5.7	30	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 154	Mar. 6	2.1	24	2.1	24	4.2	25	2.3	21	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 166	Mar. 23	4.7	31	4.3	30	7.0	30	2.7	..	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 178	April 6	7.1	29	6.4	34	8.7	38	2.2	31	Negative rods	....	....	....	....	....	....	....	....	0.1	Few
B 190	April 22	5.2	37	5.7	35	9.0	34	5.8	41	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 202	May 7	5.7	26	4.5	31	7.0	31	4.0	30	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 214	May 23	4.2	..	6.0	30	7.7	29	7.8	33	Negative rods	+	+	o	Acid	Full	+	o	Acid	Full	+
B 226	June 8	4.3	26	5.6	27	8.9	33	4.8	31	Negative rods	+	+	o	Acid	Full	+	o	Acid	Full	+
B 238	June 25	6.3	34	5.8	36	7.4	35	6.5	26	Negative rods	5.0	+	o	Acid	Full	+	o	Acid	Full	+
B 250	July 13	6.3	33	6.0	38	7.7	..	3.0	33	Negative rods	4.5	+	....	....	Full	+	o	Acid	Full	+
B 262	July 27	7.3	37	6.3	31	8.4	35	5.9	30	Negative rods	6.1	+	o	Acid	Full	+	+	Acid	0.0	o
Average		5.4	31	4.8	30	7.3	32	4.7	31											
Minimum		2.1	24	1.7	18	3.2	17	2.2	21											
Maximum		7.3	37	7.2	38	9.1	39	11.2	41											

TABLE 12—*Continued.*

SUBJECT D.

Laboratory No.	Date	Inoculated with Mixed Fecal Flora, Unheated													Inoculated with Spore Material, Heated at 80° C. for 15 Minutes					
		Sugar Broth									Litmus Milk				Litmus Milk				Blood Broth	
		Dextrose		Levulose		Lactose		Saccharose		Predominant Bacteria in Gram-stained Sediments	Gas Cms.	Coagulation	Digestion	Reaction	Gas Cms.	Coagulation	Digestion	Reaction	Gas Cms.	Capsules
		Gas Cms.	CO_2 %	Gas Cms.	CO_2 %	Gas Cms.	CO_2 %	Gas Cms.	CO_2 %											
B 34	Nov. 11	2.4	33	2.2	31	4.0	37	2.9	31	.....	....	....	....	....	....	....	....	....	....	....
B 57	Nov. 23	3.5	29	2.0	20	3.7	30	3.3	30	.....	....	....	....	....	....	....	....	....	....	....
B 80	Dec. 10	2.9	24	2.5	20	4.8	27	2.9	24	.....	....	....	....	....	....	....	....	....	....	....
B 99	Dec. 23	3.8	29	3.1	19	3.9	50	2.3	35	.....	....	....	....	....	....	....	....	....	....	....
B 112	Jan. 9	3.3	21	4.0	20	4.6	28	5.7	33	.....	1.2	+	o	Acid	....	....	....	....	....	....
B 123	Jan. 25	....	..	5.0	30	5.4	27	5.0	30	.....	2.0	+	o	Acid	....	....	....	....	....	....
B 135	Feb. 10	3.1	16	6.6	34	5.6	32	5.8	34	Negative cocci	0.5	+	o	Acid	....	....	....	....	....	....
B 147	Feb. 27	3.8	31	1.1	18	7.3	28	2.9	24	Positive cocci	....	....	....	....	....	....	....	....	5.3	o
B 157	Mar. 11	4.0	17	3.6	27	6.0	23	1.2	16	Mixed	....	....	....	....	....	....	....	....	Full	o
B 169	Mar. 27	5.7	36	6.0	36	6.6	40	4.7	36	Mixed	....	....	....	....	....	....	....	....	Full	o
B 181	April 10	5.7	31	4.5	35	6.0	41	5.7	35	Positive cocci	....	....	....	....	....	....	....	....	0.0	o
B 193	April 27	5.2	34	5.5	38	8.3	33	5.0	40	Positive cocci	....	....	....	....	....	....	....	....	0.0	o
B 205	May 13	3.0	26	3.3	24	2.8	25	3.3	24	Positive cocci	1.0	+	o	Acid	(Unch	anged	after 3	days)	0.0	o
B 217	May 28	4.0	27	3.2	28	5.5	27	4.8	31	Positive cocci	3.0	+	o	Acid		Uncha	nged		0.0	o
B 229	June 15	4.9	30	2.9	20	4.5	24	1.7	23	Negative rods	2.5	+	o	Acid		Uncha	nged		0.0	o
B 241	July 1	3.0	30	4.4	31	5.3	32	3.5	28	Mixed	3.5	+	o	Acid		Uncha	nged		0.0	o
B 253	July 17	4.2	33	4.0	25	6.2	33	5.5	34	Positive cocci	3.0	+	o	Acid	7.8	+	+	Acid	9.0	+
B 265	July 31	1.7	17	0.5	..	5.8	32	6.2	33	Mixed	3.0	+	o	Acid	Full	+	o	Acid	Full	+
Average		4.0	29	3.8	27	5.7	33	4.3	30											
Minimum		1.7	16	0.5	18	2.8	23	1.2	16											
Maximum		5.7	36	6.6	36	8.3	50	6.2	40											

SUBJECT E.

B 41.....	Nov. 14	3.7	30	3.5	34	6.1	43	3.7	30	.....	....	....	....	....	....	....	....	....	....	....
B 64.....	Nov. 27	5.1	33	2.8	21	8.1	38	4.3	32	.....	....	....	....	....	....	....	....	....	....	....
B 87.....	Dec. 13	7.0	21	3.0	23	9.0	33	2.3	17	.....	....	....	....	....	....	....	....	....	....	....
B 106.....	Dec. 30	2.5	24	2.8	21	7.6	30	3.0	26	.....	+	+	o	Acid	....	....	....	....	....	....
B 118.....	Jan. 16	3.5	28	3.6	28	8.9	28	5.1	27	Mixed	1.0	+	o	Acid	....	....	....	....	....	....
B 130.....	Jan. 31	9.6	33	8.2	33	8.3	38	4.8	29	Negative rods	0.5	+	o	Acid	....	....	....	....	....	....
B 142.....	Feb. 19	5.7	31	4.3	19	6.3	33	5.3	32	Negative rods	2.0	+	o	Acid	....	....	....	....	Full	+
B 149.....	Mar. 2	1.8	16	1.7	18	3.9	30	3.0	26	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 161.....	Mar. 16	4.1	32	....	..	3.5	34	2.1	30	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 173.....	April 1	6.1	34	5.2	36	5.7	38	5.2	25	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 185.....	April 16	6.6	33	6.6	35	7.3	33	6.2	38	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 197.....	May 1	6.8	33	5.5	36	8.4	40	6.9	30	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 209.....	May 18	4.4	31	4.5	27	5.7	31	4.5	29	Negative rods	0.5	+	o	Acid	Full	+	o	Acid	Full	+
B 221.....	June 3	5.4	31	5.1	30	7.6	30	4.1	26	Negative rods	2.0	+	o	Acid	Full	+	o	Acid	Full	+
B 233.....	June 19	5.5	35	5.5	32	8.0	38	8.2	33	Negative rods	8.3	+	o	Acid	Full	+	o	Acid	Full	+
B 245.....	July 6	8.2	37	7.0	40	7.1	35	7.4	31	Mixed	Full	+	o	Acid	Full	+	o	Acid	Full	+
B 257.....	July 22	4.8	31	5.1	35	6.2	32	5.8	33	Mixed	8.1	+	o	Acid	Full	+	+	Acid	Full	+
Average		5.3	30	4.6	29	6.9	34	4.8	29											
Minimum....		1.8	16	1.7	18	3.5	28	2.1	17											
Maximum ...		9.6	37	8.2	40	9.0	43	8.2	38											

SUBJECT F.

B 38.....	Nov. 13	1.3	30	1.4	21	2.2	27	4.6	34	.....	....	....	....	....	....	....	....	....	....	....
B 61.....	Nov. 26	2.7	33	4.9	28	2.9	31	4.0	32	.....	....	....	....	....	....	....	....	....	....	....
B 84.....	Dec. 12	1.4	..	2.4	..	1.7	..	2.3	..	.....	....	....	....	....	....	....	....	....	....	....
B 103.....	Dec. 28	3.0	27	2.6	19	4.1	36	4.3	35	Negative rods	....	....	....	....	....	....	....	....	....	....
B 115.....	Jan 14	5.2	23	4.4	27	4.7	19	2.3	26	Negative cocci	3.4	+	o	Acid	....	....	....	....	....	....
B 127.....	Jan. 29	7.4	32	4.5	24	4.6	34	5.0	17	Mixed	1.8	+	o	Acid	....	....	....	....	....	....
B 139.....	Feb. 17	4.5	22	5.3	22	6.2	29	4.3	30	Negative rods	0.5	+	o	Acid	.....	....	....	....	Full	....
B 153.....	Mar. 6	1.8	22	....	..	1.3	15	4.3	21	Positive cocci	....	....	....	....	....	....	....	....	Full	?
B 165.....	Mar. 23	4.3	27	4.3	28	5.2	28	4.9	38	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 177.....	April 6	4.9	32	5.0	30	5.8	36	4.3	39	Negative rods	....	....	....	....	....	....	....	....	Full	....
B 189.....	April 22	5.7	35	4.6	30	7.0	37	5.6	41	Positive cocci	....	....	....	....	....	....	....	....	5.6	o
B 201.....	May 7	4.3	30	4.2	31	7.0	40	4.3	30	Negative rods	....	....	....	....	....	....	....	....	Full	o
B 213.....	May 23	4.2	23	3.3	27	3.7	24	5.5	34	Positive cocci	3.0	+	o	Acid	Full	+	o	Acid	Full	+
B 225.....	June 8	2.0	15	2.0	25	2.9	20	1.9	21	(Not stained)	3.0	+	o	Acid	Full	+	o	Acid	Full	+
B 237.....	June 25	5.8	31	6.1	32	7.6	34	4.2	33	Mixed	4.0	+	o	Acid	Full	+	o	Acid	Full	+
B 249.....	July 13	4.1	29	5.1	33	6.8	35	5.4	35	Negative rods	0.0	+	o	Acid	0.0	Un	chang	ed	0.0	o
B 261.....	July 27	4.3	30	4.5	26	4.9	28	6.4	35	Mixed	2.5	+	o	Acid	0.0	Un	chang	ed	0.0	o
Average.....		3.9	28	4.0	27	4.6	30	4.3	31											
Minimum....		1.3	15	1.4	19	1.3	15	1.9	17											
Maximum ...		7.4	35	6.1	33	7.6	40	6.4	41											

TABLE 12—*Continued.*

SUBJECT G.

Laboratory No.	Date	Inoculated with Mixed Fecal Flora, Unheated												Inoculated with Spore Material, Heated at 80° C. for 15 Minutes					
		Sugar Broth								Litmus Milk				Litmus Milk				Blood Broth	
		Dextrose		Levulose		Lactose		Saccharose		Predominant Bacteria in Gram-stained Sediments	Gas Cms.	Coagulation	Digestion	Reaction	Gas Cms.	Coagulation	Digestion	Reaction	Gas Cms.	Capsules
		Gas Cms.	CO_2 %	Gas Cms.	CO_2 %	Gas Cms.	CO_2 %	Gas Cms.	CO_2 %											
B 42	Nov. 15	...	..	...	..	6.1	34	2.2	23	.....	....	....	....	....	....	....	....	....	....	....
B 65	Nov. 29	4.2	26	4.7	26	9.5	29	3.3	27	.....	....	....	....	....	....	....	....	....	....	....
B 88	Dec. 14	2.3	22	0.8	13	3.0	27	3.3	24	.....	....	....	....	....	....	....	....	....	....	....
B 107	Jan. 2	9.7	35	3.4	26	6.3	28	8.0	36	.....	+	+	o	Acid	....	....	....	....	....	....
B 119	Jan. 20	6.6	33	6.9	34	7.4	32	11.8	33	Negative rods	6.9	+	o	Acid	....	....	....	....	....	....
B 131	Feb. 3	8.8	28	8.2	30	10.5	34	9.9	29	Negative rods	Ful l	+	o	Acid	....	....	....	....	....	....
B 143	Feb. 21	5.4	31	4.1	31	8.0	35	4.7	32	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 152	Mar. 4	2.5	20	3.8	21	7.5	36	3.1	26	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 164	Mar. 19	4.1	24	...	..	8.5	30	4.7	25	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 176	April 3	7.2	38	4.7	34	6.2	32	7.0	34	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 188	April 20	6.6	39	5.8	32	9.3	45	5.7	36	Mixed	....	....	....	....	....	....	....	....	Full	+
B 200	May 4	5.9	34	4.0	33	8.6	44	2.5	32	Mixed	....	....	....	....	....	....	....	....	Full	+
B 212	May 20	7.4	26	4.8	30	7.3	31	4.5	33	Mixed	....	....	....	....	....	....	....	....	Full	+
B 224	June 5	7.2	32	5.0	28	3.0	33	6.5	30	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 236	June 22	10.0	35	9.0	30	10.7	33	5.2	36	Mixed	8.6	+	o	Acid	Full	+	o	Acid	Full	+
B 248	July 9	4.4	31	2.5	24	6.9	32	4.5	31	Mixed	3.3	+	o	Acid	Full	+	+	Acid	Full	+
B 264	July 29	4.9	32	3.3	27	10.3	34	6.7	34	Positive cocci	9.0	+	o	Acid	Full	+	+	Acid	Full	+
Average		6.1	30	4.7	28	7.6	32	5.5	31											
Minimum		2.3	20	0.8	13	3.0	27	2.2	23											
Maximum		10.0	39	9.0	34	10.7	45	11.8	36											

SUBJECT H.

B 48	Nov. 18	3.0	27	4.5	36	5.8	36	4.6	33	.....	....	....	....	....	....	....	....	....	....	....
B 71	Dec. 3	4.5	20	3.0	27	4.6	30	1.1	18	.....	....	....	....	....	....	....	....	....	....	....
B 94	Dec. 19	3.9	28	3.1	25	6.9	36	1.3	23	.....	....	....	....	....	....	....	....	....	....	....
B 109	Jan. 6	8.0	30	4.3	30	7.7	29	5.2	30	Negative rods	+	+	o	Acid	....	....	....	....	....	....
B 121	Jan. 22	6.4	29	5.1	29	5.3	21	9.9	33	Negative rods	3.0	+	o	Acid	....	....	....	....	....	....
B 133	Feb. 6	7.4	33	6.1	37	6.1	34	5.2	34	Negative rods	1.0	+	o	Acid	....	....	....	....	....	....
B 145	Feb. 24	5.0	30	...	..	8.2	25	7.1	28	Positive cocci	....	....	....	....	....	....	....	....	Full	....
B 156	Mar. 10	6.4	32	3.5	..	5.0	30	7.3	23	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 168	Mar. 25	6.0	46	4.8	38	7.3	37	1.5	33	Negative rods	....	....	....	....	....	....	....	....	Full	++
B 180	April 8	5.7	31	4.5	31	6.0	30	2.8	30	Negative rods	....	....	....	....	....	....	....	....	Full	+++
B 192	April 25	6.0	38	6.7	37	7.4	36	6.2	37	Mixed	....	....	....	....	....	....	....	....	Full	+++
B 204	May 11	6.1	29	4.8	29	8.5	35	6.1	29	Negative rods	....	....	....	....	Full	+	o	Acid	Full	+++
B 216	May 25	5.6	33	5.6	30	5.4	31	7.9	38	Negative rods	0.5	+	o	Acid	Full	+	o	Acid	Full	++o+
B 228	June 12	6.8	35	...	..	6.3	33	4.5	31	Negative rods	6.0	+	o	Acid	Full	+	o	Acid	o.o	
B 240	June 29	6.1	30	5.8	34	8.5	35	8.3	35	Mixed	6.5	+	o	Acid	Full	+	o	Acid	Full	
B 252	July 15	7.5	30	5.7	35	5.5	30	7.1	33	Positive rods	9.0	+	o	Acid	Full	+	+	Acid	Full	+++
B 260	July 24	6.9	33	6.2	32	7.2	32	6.3	33	Positive rods	7.8	+	o	Acid	Full	+	o	Acid	Full	++
Average		6.0	31	4.9	32	6.6	32	5.4	31											
Minimum		3.0	20	3.0	25	4.6	21	1.1	18											
Maximum		8.0	46	6.7	38	8.5	37	9.9	38											

SUBJECT I.

B 32	Nov. 11	1.4	21	1.3	15	4.1	29	3.4	38	.....	....	....	....	....	....	....	....	....	....	....
B 55	Nov. 27	...	..	4.0	20	4.7	34	3.0	23	.....	....	....	....	....	....	....	....	....	....	....
B 78	Dec. 9	5.0	10	4.8	33	5.7	35	3.7	27	.....	....	....	....	....	....	....	....	....	....	....
B 101	Dec. 26	8.0	38	2.4	25	4.8	33	3.9	38	Negative rods	....	....	....	....	....	....	....	....	....	....
B 113	Jan. 12	6.2	29	5.0	30	10.9	30	5.5	33	.....	4.0	+	o	Acid	....	....	....	....	....	....
B 125	Jan. 27	6.2	27	5.6	25	5.4	33	5.3	34	Negative rods	4.5	+	o	Acid	....	....	....	....	....	....
B 137	Feb. 14	4.2	24	4.2	29	8.4	39	6.7	34	Negative rods	2.5	+	o	Acid	....	....	....	....	Full	+
B 160	Mar. 14	3.4	20	2.7	25	5.6	32	3.9	30	Positive cocci	....	....	....	....	....	....	....	....	Full	....
B 172	Mar. 31	5.4	29	4.8	25	7.3	35	5.0	40	Positive cocci	....	....	....	....	....	....	....	....	Full	....
B 184	April 13	4.6	23	4.7	31	8.3	42	6.2	38	Negative cocci	....	....	....	....	....	....	....	....	Full	....
B 196	April 29	5.6	32	4.5	31	6.9	29	3.4	32	Positive cocci	....	....	....	....	....	....	....	....	Full	+
B 208	May 15	3.7	27	3.2	28	5.4	33	3.2	31	Positive cocci	....	....	....	....	Full	+	o	Acid	Full	....
B 220	June 1	5.2	26	4.8	31	10.9	33	5.8	36	Mixed	9.0	+	o	Acid	Full	+	o	Acid	Full	+
B 232	June 17	5.7	31	6.0	35	7.5	33	5.4	35	Positive cocci	7.0	+	o	Acid	Full	+	o	Acid	Full	+
B 244	July 3	6.7	34	5.0	32	8.6	33	5.0	32	Positive cocci	6.6	+	o	Acid	Full	+	o	Acid	Full	+
B 256	July 20	6.2	38	5.6	33	7.1	35	6.7	37	(Not stained)	5.0	+	o	Acid	Full	+	o	Acid	Full	+
Average		4.8	26	4.0	26	6.6	32	4.5	32											
Minimum		1.4	10	1.3	15	4.1	29	3.0	23											
Maximum		8.0	38	6.0	35	10.9	42	6.7	40											

TABLE 12—*Continued.*

SUBJECT J.

Laboratory No.	Date	Inoculated with Mixed Fecal Flora, Unheated												Inoculated with Spore Material, Heated at 80° C. for 15 Minutes					
		Sugar Broth								Litmus Milk				Litmus Milk				Blood Broth	
		Dextrose		Levulose		Lactose		Saccharose		Predominant Bacteria in Gram-stained Sediments	Gas Cms.	Coag-ulation	Diges-tion	Reac-tion	Gas Cms.	Coag-ulation	Diges-tion	Reac-tion	Gas Cms.	Cap-sules
		Gas Cms.	CO_2 %	Gas Cms.	CO_2 %	Gas Cms.	CO_2 %	Gas Cms.	CO_2 %											
B 31	Nov. 9	3.1	29	1.3	15	4.9	31	3.1	32	.....	....	....	....	....	....	....	....	....	....	....
B 54	Nov. 21	2.1	24	2.3	22	2.5	24	3.4	32	Negative rods	....	....	....	....	....	....	....	....	....	....
B 77	Dec. 7	1.6	12	2.1	24	4.9	35	3.7	22	.....	....	....	....	....	....	....	....	....	....	....
B 100	Dec. 23	4.7	15	3.1	3	4.0	5	2.4	38	Negative rods	....	....	....	....	....	....	....	....	....	....
B 111	Jan. 9	1.6	31	5.8	32	5.2	29	2.4	25	Negative rods	?	+	o	Acid	....	....	....	....	....	....
B 124	Jan. 25	8.2	28	7.4	33	6.3	33	11.6	25	Negative rods	?	+	o	Acid	....	....	....	....	....	....
B 136	Feb. 10	7.1	27	6.2	30	5.3	37	2.7	22	Negative rods	1.0	+	o	Acid	....	....	....	....	....	....
B 148	Feb. 27	2.6	24	1.5	..	6.4	28	3.4	27	Negative rods	....	....	....	....	....	....	....	....	0.2	o
B 159	Mar. 13	5.7	25	5.6	26	6.9	33	5.2	35	Negative rods	....	....	....	....	....	....	....	....	4.0	o
B 171	Mar. 30	6.3	33	5.5	25	6.6	21	3.1	32	Negative rods	....	....	....	....	....	....	....	....	3.1	o
B 183	April 14	6.5	35	6.0	30	6.2	30	6.7	40	Negative rods	....	....	....	....	....	....	....	....	0.0	o
B 195	April 29	6.2	39	5.5	32	6.4	37	7.2	37	Negative rods	....	....	....	....	....	....	....	....	0.0	o
B 207	May 15	4.0	..	3.6	26	5.3	30	3.8	29	Negative rods	....	....	....	....	0.0	o	o	Alk.	0.0	o
B 219	June 1	6.7	30	5.7	26	7.6	31	7.2	34	Negative rods	7.0	+	o	Acid	0.0	....	....	Alk.	6.1	o
B 231	June 17	3.7	22	4.1	22	5.0	20	6.9	20	Negative rods	4.6	+	o	Acid	0.0	....	....	Alk.	0.0	o
B 243	July 4	2.9	34	2.6	23	4.3	32	5.6	39	Positive cocci	3.7	+	o	Acid	Full	+	+	Acid	Full	+
B 255	July 20	1.7	30	4.7	30	7.0	30	...	..	Positive cocci	3.5	+	o	Acid	0.0	....	+	?	0.0	o
Average		4.4	27	4.3	25	5.9	29	4.9	31											
Minimum		1.6	12	1.3	3	2.5	5	2.4	22											
Maximum		8.2	39	7.4	33	7.6	37	11.6	40											

SUBJECT K.

B 40.....	Nov. 14	1.1	18	1.3	23	2.6	30	4.4	34	.....	....	....	....	....	....	....	....	....	....	....
B 63.....	Nov. 27	4.0	30	4.2	29	8.1	38	4.3	30	.....	....	....	....	....	....	....	....	....	....	....
B 86.....	Dec. 13	6.1	34	3.5	28	5.1	29	4.2	33	.....	....	....	....	....	....	....	....	....	....	....
B 105.....	Dec. 30	10.4	26	8.3	32	7.4	33	9.3	30	.....	+	+	o	Acid	....	....	....	....	....	....
B 117.....	Jan. 16	6.3	34	5.5	29	9.7	27	10.3	38	Positive rods	Full	+	o	Acid	....	....	....	....	....	....
B 129.....	Jan. 31	8.5	31	8.2	35	7.5	33	10.7	34	Mixed	2.5	+	o	Acid	....	....	....	....	....	....
B 141.....	Feb. 19	6.2	27	4.4	38	6.5	27	6.6	36	Mixed	0.5	+	o	Acid	....	....	....	....	Full	....
B 151.....	Mar. 4	2.6	34	2.9	27	6.7	25	2.9	27	Mixed	....	....	....	....	....	....	....	....	3.2	....
B 163.....	Mar. 19	5.0	30	...	..	9.5	35	...	..	Positive rods	....	....	....	....	....	....	....	....	Full	....
B 175.....	April 3	5.1	23	5.5	43	7.9	40	2.6	34	Mixed	....	....	....	....	....	....	....	....	Full	....
B 187.....	April 20	5 6	37	6.7	40	8.5	30	8.5	41	Mixed	....	....	....	....	....	....	....	....	Full	+
B 199.....	May 4	5.3	33	2.8	21	7.5	38	3.1	25	Positive cocci	....	....	....	....	....	....	....	....	Full	+
B 211.....	May 20	9.5	28	6.7	32	9.4	35	7.0	35	Negative rods	....	....	....	....	Full	....	....	....	Full	....
B 223.....	June 5	10.2	29	9.2	35	8.8	29	7.2	31	Negative rods	....	....	....	....	....	....	....	....	Full	+
B 235.....	June 23	11.2	33	5.5	36	10.8	31	8.4	34	Positive rods	8.0	+	o	Acid	Full	+	o	Acid	Full	+
B 247.....	July 9	4.3	32	4.4	31	6.3	38	6.0	35	Positive cocci	4.0	+	o	Acid	Full	+	o	Acid	Full	+
B 263.....	July 29	4.4	31	0.6	16	6.6	34	6.0	36	Positive cocci	Full	+	o	Acid	7.0	+	o	Acid	Full	+
Average.....		6.2	30	5.0	31	8.1	32	6.3	33											
Minimum....		1.1	18	0.6	16	2.6	25	2.6	25											
Maximum ...		11.2	37	9.2	43	10.8	40	10.7	41											

SUBJECT L.

B 74.....	Dec. 6	2.4	41	10.3	30	1.9	31	1.9	21	.....	....	....	....	....	....	....	....	....	....	....
B 97.....	Dec. 20	5.3	28	3.6	47	3.2	40	9.2	40	Negative rods	....	....	....	....	....	....	....	....	....	....
B 108.....	Jan. 2	8.6	31	5.9	35	5.4	27	9.9	31	.....	+	+	o	Acid	....	....	....	....	....	....
B 120.....	Jan. 20	4.5	27	4.0	27	5.5	34	3.3	24	Negative rods	+	+	o	Acid	....	....	....	....	....	....
B 132.....	Feb. 3	5.5	30	4.2	19	5.4	33	7.3	34	Negative rods	2.8	+	o	Acid	....	....	....	....	....	....
B 144.....	Feb. 21	2.7	18	2.5	..	4.0	18	4.0	30	Positive cocci	....	....	....	....	....	....	....	....	Full	....
B 155.....	Mar. 9	0.9	33	0.4	12	2.7	22	4.1	29	Positive cocci	....	....	....	....	....	....	....	....	Full	+
B 167.....	Mar. 25	2.6	26	1.6	25	6.2	33	5.0	40	Mixed	....	....	....	....	....	....	....	....	Full	....
B 179.....	April 8	4.6	32	3.7	29	5.0	28	2.1	28	Positive cocci	....	....	....	....	....	....	....	....	0.2	o
B 191.....	April 24	2.1	23	1.6	18	8.7	21	1.3	23	Positive cocci	....	....	....	....	....	....	....	....	Full	....
B 203.....	May 11	3.7	21	2.9	20	4.5	28	2.6	23	Positive cocci	....	....	....	....	Full	+	o	Acid	Full	+
B 215.....	May 25	2.6	23	2.3	21	2.5	24	2.7	25	Positive cocci	0.1	+	o	Acid	Full	+	o	Acid	Full	+
B 227.....	June 12	3.2	28	5.6	37	9.0	38	6.0	33	Mixed	3.2	+	o	Acid	Full	+	o	Acid	Full	+
B 239.....	June 29	4.4	31	4.5	31	7.8	37	3.2	31	Positive cocci	2.4	+	o	Acid	Full	+	o	Acid	Full	+
B 251.....	July 15	7.0	30	5.4	35	7.4	36	5.6	33	Mixed	7.5	+	o	Acid	Full	+	+	Acid	Full	+
B 259.....	July 25	5.4	31	3.5	31	6.5	35	4.1	31	Mixed	3.6	+	o	Acid	Full	+	o	Acid	Full	+
Average.....		4.1	28	3.9	28	5.4	30	4.5	30											
Minimum....		0.9	18	0.4	12	1.9	18	1.3	21											
Maximum ...		8.6	41	10.3	47	9.0	40	9.9	40											

some bacteria of both groups. The small gas production occurring in some instances cannot, therefore, be ascribed to the lack of aerogenic organisms, nor is the diminished gas production due to a loss of the gas producing function in these aerogenic species, for mere pasteurization of mixt fecal flora restores the gas producing power so that milk and blood broth fermentation tubes inoculated with it become entirely filled with gas. The explanation is to be sought rather in the antagonistic action of other members of the mixt fecal flora, which are able to develop in the sugar broth fermentation tubes and to inhibit or limit the proliferation of the gas formers. One important feature of the fermentation tube cultures is the variety

TABLE 13.

Summary of Gas Production in Sugar Broth Fermentation Tubes.

		Subject A	Subject B	Subject C	Subfect D	Subject E	Subject F	Subject G	Subject H	Subject I	Subject J	Subject K	Subject L	All Subjects
Dextrose	Maximum	12.8	6.8	7.3	5.7	9.6	7.4	10.0	8.0	8.0	8.2	11.2	8.6	12.8
	Mean	**7.3**	**4.4**	**5.4**	**4.0**	**5.3**	**3.9**	**6.1**	**6.0**	**4.8**	**4.4**	**6.2**	**4.1**	**5.2**
	Minimum	4.8	2.0	2.1	1.7	1.8	1.3	2.3	3.0	1.4	1.6	1.1	0.9	0.9
Levulose	Maximum	10.5	5.7	7.2	6.6	8.2	6.1	9.0	6.7	6.0	7.4	9.2	10.3	10.5
	Mea 1	**5.5**	**4.1**	**4.8**	**3.8**	**4.6**	**4.0**	**4.7**	**4.9**	**4.0**	**4.3**	**5.0**	**3.9**	**4.5**
	Minimum	2.5	1.3	1.7	0.5	1.7	1.4	0.8	3.0	1.3	1.3	0.6	0.4	0.4
Lactose	Maximum	13.3	9.3	9.1	8.3	9.0	7.6	10.7	8.5	10.9	7.6	10.8	9.0	13.3
	Mean	**7.8**	**6.5**	**7.3**	**5.7**	**6.9**	**4.6**	**7.6**	**6.6**	**6.6**	**5.9**	**8.1**	**5.4**	**6.6**
	Minimum	3.2	3.3	3.2	2.8	3.5	1.3	3.0	4.6	4.1	2.5	2.6	1.9	1.3
Saccharose	Maximum	11.4	11.5	11.2	6.2	8.2	6.4	11.8	9.9	6.7	11.6	10.7	9.0	11.8
	Mean	**5.2**	**4.6**	**4.7**	**4.3**	**4.8**	**4.3**	**5.5**	**5.4**	**4.5**	**4.9**	**6.3**	**4.5**	**4.9**
	Minimum	0.4	1.4	2.2	1.2	2.1	1.9	2.2	1.1	3.0	2.4	2.6	1.3	0.4

of species brought to development, where the agar and gelatin plates show only *B. coli*. This is due not only to the liquid nature of the medium but to the anaerobic condition of the closed arm and also possibly to the opportunity for more intimate symbiosis afforded to the mixt bacteria, resembling in a measure the conditions of growth in the upper part of the intestine. It would be, however, a serious error to assume that the fermentation tube furnishes the same conditions as the intestine or that it allows the development of all species of fecal bacteria, or favors equally the multiplication of those species which do grow in it. It is merely an empirical method of demonstrating the presence of some living bacterial species which might otherwise be missed, and of studying the associated action of the fecal bacteria upon the liquid media. The kind of fermentation set up,

and the relative numbers of the different types of bacteria found in the sediment may, when some reliable means of interpretation has been furnished, prove of some value as an index of the number and activity of these different species in the intestine.

In Table 12 are also given the rather fragmentary results of some special tests for the immediate recognition of *B. welchii*, our Type I, and for the Type VIII (and IX), which completely digests milk. Litmus milk fermentation tubes, inoculated with 0.25 c.c. of Suspension 1, became coagulated with an acid reaction in every instance. The gas production was usually less than half the closed arm, but there are several instances in which the closed arm was filled with gas, indicating a marked development of *B. welchii*. No digestion of the casein was observed in these tubes. Litmus milk fermentation tubes inoculated with 0.50 c.c. of Suspension No. 2, Spores, usually became coagulated with an acid reaction and the closed arm of the tube entirely filled with the gas, indicating the unobstructed action of the gas bacillus. In a few instances the casein was digested without acid production and without the formation of gas, indicating the presence of Type VIII (or IX), and in some tubes there was evidence of the associated action of two or more sporogenic species. A comparison of the behavior of these two sets of milk fermentation tubes, the one inoculated with the unheated mixt fecal flora and the other with the same material after it had been heated to 80° C. for 15 minutes, indicates the marked influence which the asporogenic intestinal bacteria exert upon the behavior of the sporogenic forms.

The broth fermentation tube to which has been added a bit of naturally sterile liver or other animal tissue, as recommended by Smith,[13] was found to be especially useful in the rapid detection of *B. welchii*. Instead of the solid tissue we have usually added naturally sterile defibrinated blood to the broth, which appears to be equally serviceable. These tubes inoculated with 0.50 c.c. of Suspension No. 2, Spores, usually became filled with gas within 24 hours at 37° C., and the capsulated bacilli could be readily demonstrated by suitable staining of the sediments. This is the easiest and simplest culture method for recognizing the presence of the gas bacillus spores in feces.

CONCLUSIONS.

1. The number of fecal bacteria brought to development on artificial culture media is a minute fraction of the total fecal bacteria microscopically visible.

2. Upon aerobic lactose gelatin plates, upon aerobic lactose agar, and anaerobic glucose agar plates, bacteria of the *B. coli* group are the only members of the normal fecal flora brought to development in a satisfactory manner.

3. Sporogenic aerobic bacilli are normally present in the human feces, and one species has been repeatedly isolated from each of the 12 individuals.

4. Sporogenic anaerobic bacilli may be isolated from nearly every sample of human feces, by appropriate methods. *B. welchii* is a normal constituent of the fecal flora.

5. The quantity of vegetative cells of the sporogenic species has not been estimated. The total number of sporogenic bacilli in the feces is therefore probably much greater than indicated by the cultures of spore material.

6. Among the visible bacteria which fail to grow, a considerable number are undoubtedly dead cells belonging to cultivable species. The death of these cells is primarily due to the unfavorable products of their own growth, the effect of which is exaggerated by the concentration of intestinal contents, the absorption of food material, and the antagonistic action of other bacterial species.

7. The variation in the number of cultivable *B. coli* in the normal feces is largely dependent upon the extent to which this bacterial destruction has progressed.

8. There are a number of bacterial species in normal feces, not yet brought to satisfactory development on plate cultures.

9. The fermentation tube filled with various liquid media is exceedingly valuable in bringing to development various species of fecal bacteria.

10. The gas produced in sugar broth fermentation tubes, inoculated with the mixt bacteria contained in 0.0025 gm. feces, is fairly constant in composition, but exceedingly variable in quantity.

11. This variation in the amount of gas produced is not necessarily due to an alteration in the aerogenic function of the gas-forming

fecal bacteria, but is due rather to variations in the extent to which other species develop in the fermentation tubes and restrict the action of the gas formers.

In conclusion it is a pleasure again to acknowledge the encouragement of Professor H. S. Grindley, chief of the laboratory, and the assistance of Professor Theobald Smith, whose counsel has been of especial value to us in this part of the work.

REFERENCES.

1. BELONOWSKI. *Russky Wratsch*, 1907, No. 21, p. 720. Ref. in *Jahresb. ü. d. Fortschr. der Thier-Chemie*, 1907, 37, p. 392.
2. BIENSTOCK. *Ztschr. f. klin. Med.*, 1884, 8, pp. 1–43; *Ann. de l'Inst. Pasteur*, 1906, 20, pp. 407–15.
3. CONRADI UND KURPJUWEIT. *Münch. med. Wchnschr.*, 1905, 52, pp. 1761–64; *ibid.*, 1905, 52, pp. 2164–68; 2228–32.
4. EIJKMAN. *Centralbl. f. Bakt.*, 1904, Abt. 1, Orig., 37, pp. 436–49.
5. ESCHERICH. *Die Darmbakterien des Säuglings, u. s. w.* Enke, Stuttgart, 1886.
6. GESSNER. *Archiv f. Hyg.*, 1889, 9, pp. 128–38.
7. HEHEWERTH. *Archiv f. Hyg.*, 1901, 39, pp. 321–89.
8. HERTER. *The Common Bacterial Infections of the Digestive Tract.* New York, 1907.
9. HERTER. *U. S. Dept. Agr. Report No. 88*, 1909, pp. 565–768.
10. KLEIN. *Archiv f. Hyg.*, 1902, 45, pp. 117–76.
11. KOLLE UND WASSERMANN. *Handbuch der Pathogenen Mikroorganismen.* Jena, 1903–4.
12. SCHMIDT UND STRASBURGER. *Die Faeces des Menschen.* Berlin, 1905.
13. SMITH, BROWN, AND WALKER. *Jour. Med. Res.*, 1905, 14, pp. 193–206.
14. STERN. *Ztschr. f. Hyg.* 1892, 12, pp. 88–136.
15. SUCKSDORFF. *Archiv f. Hyg.*, 1886, 4, pp. 355–96.
16. TAVEL. *Centralbl. f. Bakt.*, Abt. 1, Orig., 1898, 23, pp. 538–41.
17. TISSIER. *Recherches sur la Flore Intestinale des Nourrissons.* Thèse, Paris, 1900.
18. ———. *Ann. de l'Inst. Pasteur*, 1905, 19, pp. 109–23.
19. ———. *Ann. de l'Inst. Pasteur*, 1908, 22, pp. 189–208.
20. WELCH AND NUTTALL. *Bull. Johns Hopkins Hosp.*, 1892, 3, pp. 81–91.